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VOLUME 61 OF 78 OF SUBMISSION

CGA-152005

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STUDY TITLE

ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING INCLUDING VALIDATION DATA SUPERCEDES AG-590

DATA REQUIREMENT

EPA GUIDELINE NUMBER 171-4(c)

STUDY DIRECTOR

ROBERT E. M. WURZ, Ph.D.

STUDY COMPLETED

DECEMBER 15, 1993

PERFORMING LABORATORY

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LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD AG-590A

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 87

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B) or (C).

Company: Ciba Plant Protection, Ciba-Geigy Corporation

Company Representative: Thomas J. Parshley

Title: Senior Regulatory Manager

Thomas J Parshley
Signature

1/19/94
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STATEMENT CONCERNING GOOD LABORATORY PRACTICE

The Good Laboratory Practices Compliance Statement regarding EPAs GLP Standards (40 CFR Part 160) provided on page twenty-one (21) of this volume and signed by the Study Director is truthful and accurate.

W.T. Beidler — 1-19-94
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ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING INCLUDING VALIDATION DATA

ANALYTICAL METHOD NO. AG-590A
(Supercedes AG-590)

PROJECT NUMBER: 168982

SUBMITTED BY: R. E. M. Wurz TITLE: Research Scientist

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I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for determination of residues of CGA-152005 in crops and crop fractions. The limit of detection of this method, determined by the smallest standard amount injected, is 0.8 ng of CGA-152005. The limit of determination as determined by fortification experiments is 0.01 ppm. The chemical names and structures of CGA-152005 are shown in Figure 1.

This method supercedes Analytical Method AG-590[1], and contains no new data. The purpose of AG-590A is to expand the modifications and potential problems section to include helpful information gathered during a PR 88-5 ruggedness trial[2]. AG-590 still stands as the final report for the method validation study.

B. PRINCIPLE

A 6-g subsample of crop substrate is homogenized twice with acetonitrile (ACN/aqueous sodium bicarbonate. Both extracts are filtered through glass wool and combined. A 150-ml aliquot of extract is transferred to a flask and the volume reduced to <0.5 ml. The concentrated extract is diluted with saturated sodium chloride solution and sodium carbonate solution and partitioned against methyl tert-butyl ether (MTBE)/hexane. The aqueous solution is retained and acidified with dilute phosphoric acid before being loaded onto a 20-ml ChemElut (or Extrelute) cleanup column. The sample on the ChemElut column is partitioned with dichloromethane (DCM)/hexane and the organic solution is collected. The sample solution is evaporated to incipient dryness and the residue reconstituted in ACN/aqueous ammonium hydroxide. Residue determination is done by narrow bore HPLC with column switching (250 x 2.0 mm Cyano column to a 250 x 2.1 mm Supelcosil LC-18-DB column) with UV detection at 225 nm. See Figure 3 for analytical flowchart.

Oil samples (5-g) are dissolved in 50 ml of hexane and partitioned with carbonate solution. The aqueous layer is diluted with saturated sodium chloride solution and back partitioned with hexane before being acidified and taken to

the ChemElut column as above. See Figure 4 for analytical flowchart.

II. MATERIALS AND METHODS

A. APPARATUS

1.0 Bottles, square amber wide mouth, 8 oz.

2.0 Bottles, Boston Round, narrow mouth, 8 oz.

3.0 Erlenmeyer flask, 125-ml 250-ml

4.0 Carbon filter tube

5.0 Concentration tube, minimum volume 25-ml

6.0 Disposable Pasteur pipets

7.0 Funnel, long stem, 12.5-cm size

8.0 Funnel, powder, 80-mm

9.0 Funnel, separatory, 60-ml and 125-ml with Teflon stopcock

10.0 Glass wool

11.0 Graduated cylinder, 50-ml, 100-ml or equivalent

12.0 Homogenizer, Polytron or equivalent

13.0 Round bottom flasks, 500-ml, 250-ml

14.0 Rotary evaporator, Buchii or equivalent

15.0 Syringe filter, ACRODISC LC13 PVDF, 0.2 µm (Gelman #4450)

16.0 Stopcock, 2-way, nylon (ISOLAB, Inc. #QSV)

17.0 Syringe, glass multifit 2 and 5-cc size

18.0 Vials, Wheaton, 2-ml or equivalent

19.0 Volumetric pipets, 1-ml, 2-ml, 8-ml, 10-ml

B. REAGENTS

1.0 Acetonitrile (ACN), HPLC grade

2.0 Ammonium hydroxide (NH_4OH), ACS Reagent grade

3.0 0.05% conc. NH_4OH/water (v/v)

4.0 Dichloromethane (DCM), HPLC grade

5.0 50% DCM/Hexane (v/v)

6.0 Hexane, HPLC grade

7.0 Methyl tert-butyl ether (MTBE), HPLC grade

8.0 Phosphoric acid (H_3PO_4) conc., Certified ACS grade

9.0 0.8% conc. H_3PO_4/water (v/v)

10.0 Sodium chloride, Certified ACS grade

11.0 Saturated solution of sodium chloride in water

12.0 Sodium bicarbonate, Certified ACS grade

13.0 Sodium carbonate, Certified ACS grade

14.0 0.4% Sodium carbonate/water (w/v)

15.0 8:2 ACN:0.1% Sodium bicarbonate/water (w/v)

16.0 Water, HPLC grade

17.0 ChemElut, 20-ml capacity (Varian cat. #1219-8008) or equivalent (Extrelute QE).

18.0 CGA-152005, Analytical Standard supplied by Ciba-Geigy Corporation, 410 Swing Road, Greensboro, NC 27419

C. ANALYTICAL PROCEDURES

1.0 Sample Preparation

Samples are received and stored frozen at -20°C (Ciba SOP 7.20). Samples are prepared under the general guidelines of the U.S. Food and Drug Administration Pesticide Analytical Manual Volume I, Section 141 (Ciba SOP 7.21).

2.0 Extraction

2.1 **OIL SAMPLES**: Transfer 5 g of crude or refined oil to a 125-ml flask and add 50 ml of hexane to dissolve the sample. Transfer the organic solution to a 125-ml separatory funnel. Rinse the flask with precisely 10 ml of 0.4% sodium carbonate solution and add this rinse to the separatory funnel. Gently shake the separatory funnel for 3 minutes, then allow the phases to separate (Caution: emulsions form easily). Drain the lower, aqueous phase and any remaining emulsion back into the flask and discard the upper, organic layer.

Add 10 ml of saturated sodium chloride solution to the aqueous solution in the flask and transfer the combined volumes back into the separatory funnel. Add 25 ml of hexane to the separatory funnel and shake for one minute. Allow the layers to separate, then drain the lower, aqueous layer into the 125-ml flask and carry this solution on to Section II.C.4.1. Discard the organic layer.

2.2 **CROP RAC'S AND SOLID FRACTIONS**: Weigh a 6-g aliquot of crop substrate into an 8 oz. wide mouth jar. Fortify with CGA-152005 at this point for recovery samples. Immediately add 90 ml 8:2 ACN:0.1% sodium bicarbonate/water and let the sample steep for 15 minutes. Homogenize the sample with a Polytron homogenizer at medium power for 30 seconds. Filter the sample through a plug of glass wool at the apex and stem of a carbon filter tube into an amber Boston round bottle, or Erlenmeyer flask if for immediate use. Return any crop matrix in the carbon filter tube and the glass wool to the extraction jar. Rinse any matrix residue adhering to

the carbon filter tube into the extraction jar with 90 ml 8:2 ACN:0.1% sodium bicarbonate solution.

Homogenize the sample plus glass wool and solvent again for 30 seconds and filter the extract through a new plug of glass wool at the apex and stem of the carbon filter tube. Collect both extracts in the same container and refrigerate the sample extract if it is not to be used immediately.

3.0 Partition Cleanup

3.1 Transfer an 150-ml aliquot of sample extract to a 500-ml round bottom flask and remove the solvent by rotary vacuum evaporation until the volume is <0.5 ml (bath temperature <40°C). Add 10 ml of 0.4% sodium carbonate solution to the round bottom flask and sonicate to loosen or dissolve any adhering residue. Transfer the solution to a 60-ml separatory funnel (See Section II.H.3.0 for problems with sample solution pH ranges).

3.2 Add 10 ml of saturated sodium chloride solution to the 500-ml round bottom flask and swirl. Transfer the solution to the 60-ml separatory funnel in Section II.C.3.1. Add 25 ml of 1:1 MTBE:hexane to the 500-ml round bottom flask and swirl. Transfer the solution to the 60-ml separatory funnel above.

3.3 Stopper the 60-ml separatory funnel and shake for one minute, taking care to vent the funnel. Allow the two layers to separate. Break any emulsion that may form and drain the lower, aqueous layer and any remaining emulsion back into the 500-ml round bottom flask from Section II.C.3.2. Discard the upper, organic layer and transfer the aqueous layer back to the separatory funnel.

3.4 Add 25 ml of 1:1 MTBE:hexane to the 60-ml separatory funnel, stopper and shake for one minute. Break any emulsion that may form and drain the lower, aqueous layer and any remaining emulsion back into the 500-ml round bottom flask from Section II.C.3.3. Discard the upper, organic layer.

4.0 ChemElut Cleanup

4.1 Add 8 ml of 0.8% phosphoric acid solution to the aqueous layer in the 500-ml round bottom flask from Section II.C.3.4 (or the flask from Section II.C.2.4 for oil samples) and swirl to mix. Transfer the sample solution to the 20-ml ChemElut cleanup column by passing it through (rinsing) the 60-ml separatory funnel in which the partitions were done. Let the solution sit in the ChemElut column for at least 5 minutes.

4.2 Attach a reservoir to the ChemElut column and partition the sample with 100 ml of 1:1 DCM:hexane. The flow through the ChemElut should be no greater than 2-3 ml per minute. The flow may be controlled by attaching a nylon stopcock to the outlet of the column. Collect the organic solution in a 250-ml round bottom flask. CAUTION: If any aqueous solution breaks through the ChemElut column, remove it by pipet before proceeding. No acidic aqueous solution should be present before evaporation. Evaporate the solvent from the sample solution until the volume is approximately 10 ml (water bath <35°C). Quantitatively transfer the sample solution to a concentration tube using three 2 to 3-ml acetone washes. Evaporate the sample just to dryness without any applied heat and reconstitute in the appropriate volume of 20% ACN/0.05% ammonium hydroxide solution. Sonicate and vortex stir the sample before filtering through a 0.2-µm syringe

filter into a vial for analysis by HPLC.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

1.1 Install the HPLC system according to Table I and Figure 2. Control of the switching valve is accomplished via time-programmed contact closures of the detector, autoinjector or other timing source.

1.2 Determine the retention time of CGA-152005 on Column #1 by connecting Column #1 directly to the detector and injecting 24 ng of the analyte. (Inject 40 µl of the 0.6 ng/µl standard solution prepared in Section II.I.1.0).

1.3 Connect the system as shown in Figure 2. Program the valve to switch to the INJECT POSITION at the beginning of the CGA-152005 analyte peak and to return to the LOAD POSITION at the end of the analyte peak of CGA-152005, as determined in Section II.D.1.2.

1.4 Inject 24 ng of CGA-152005 to determine its retention time through the two columns and to confirm that the valve time programming is correct.

2.0 Standardization

2.1 Calibrate the HPLC system with each analytical run by checking the retention time and detector response relative to previous runs. Retention times must not vary more than 2% within a run and detector response should not vary more than 10% between runs.

2.2 Standardize the HPLC system by injecting 40-µl aliquots of standard solutions of CGA-152005 in a working range of 0.8-24 ng/injection (Figure

5). Generate a linear regression from the data by comparing detector response and ng injected (Table II).

E. INTERFERENCES

None.

F. CONFIRMATORY TECHNIQUES

None.

G. TIME REQUIRED

A skilled analyst can complete the extraction and analysis of a set of 6-8 samples in 10-12 working hours.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Some samples may develop emulsions after shaking (Section II.C.2.3 and II.C.3.3). These may be cleared if allowed to settle out slightly and then gently stirred with a glass rod. Centrifugation can also be used to settle emulsions. It is important that the organic layer be clear of emulsion before separation. Grain samples are especially subject to loss of analyte in the uncleared organic layer during partitions. In addition, any small amounts of remaining emulsion should be taken forward through the procedures.

2.0 After fortification, samples should not stand at room temperature for a prolonged period of time before extraction.

3.0 For most samples, the pH of the aqueous solutions will be in the optimum range during the cleanup procedures. However, an occasional sample may be more acidic or basic than average, and this can lead to loss of analyte. It may necessary to check the solution pH of problematic samples at two places. In Section II.C.3.1 the pH of the sample solution should be 11 ± 1 after addition of the carbonate solution. In Section II.C.4.1, the pH of the sample solution should be 3.0 ± 1 after addition of dilute phosphoric acid. If sample solutions fall

outside the suggested pH range, then concentrated phosphoric acid or sodium hydroxide should be used for correction.

4.0 During the evaporation of sample solutions in Section II.C.4.2, any water bath used must not have a temperature >35°C and the samples should be removed as soon as they are ready. Excessive temperature, especially when the sample has gone to dryness, leads to analyte decomposition. The final evaporation to dryness must be done without external heating (during validation of this method, a vacuum centrifuge evaporator was used without applied heat, which kept the samples cold during evaporation).

5.0 Stopping Points: Refrigerated extracts have shown stability for up to 72 hours. Extract aliquots can also be evaporated to about 20-ml for overnight refrigerated storage. The hexane/DCM partition eluate may be stored refrigerated overnight prior to any evaporation.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Standard CGA-152005 Solutions

1.1 Weigh 10 mg of CGA-152005 analytical standard into a 100-ml volumetric flask and dilute to the mark with ACN.

1.2 Make serial dilutions of the 0.1 mg/ml standard solution with 20% ACN/0.05% ammonium hydroxide solution (w/v) to give a series of fortification/analytical standards in a range of 0.02 µg/ml to 3.0 µg/ml of CGA-152005. Store the standard solutions in amber bottles at 4°C in the dark when not in use. Standards have been successfully used for up to four months after preparation.

1.3 CGA-152005 is degraded in methanol. No solubility problems have been observed with CGA-152005 in the solvents used.

J. METHODS OF CALCULATIONS

1.0 Determination of Sample Residues

1.1 Inject 40-µl aliquots of sample extracts from Section II.C. into the HPLC under the same conditions as for standards. Make appropriate dilutions of the samples in 2:8 ACN:0.05% ammonium hydroxide/water solution to bring the sample peak heights within the range of the standard curve. Compare the peak heights of the unknown samples to the standard curve or enter the peak height into a least squares program to determine the nanograms of CGA-152005 in the injected aliquot. Typical chromatograms for control and procedural recovery samples are shown in Figures 6-9.

1.2 To calculate the residue results, the mg injected must first be calculated as follows: (Equation 2)

$$(2)\quad \text{mg inj.} = \frac{(G)\ (V_a)\ (V_i)}{[V_e + W(M/100)]\ (V_f)}$$

G = milligrams sample extracted
V_a = aliquot volume
V_e = extraction volume
V_i = injection volume (µl)
V_f = total volume of final injection solution (µl)
R% = recovery ratio given by equation 4
W = grams samples extracted
M = % moisture of substrate

Calculate the residue results in terms of ppm of CGA-152005 by using the following equation (R is expressed as the decimal of the percent value):

$$(1)\quad \text{ppm} = \frac{(\text{ng CGA-152005 found})}{(\text{mg sample injected})\quad (R)}$$

2.0 Fortification Experiments

This method is validated for each set of samples analyzed by including an untreated

control sample and one or more control samples fortified immediately prior to extraction with CGA-152005.

2.1 Add 1.0 ml of a 0.06 µg/ml standard solution of CGA-152005 to 6 g of control crop prior to the addition of extraction solvent for a 0.01 ppm fortification. Use correspondingly larger amounts of standards (volume should not exceed 2 ml) for higher fortifications. Analyze control and freshly fortified samples along with the treated samples according to the procedures of the method.

2.2 Calculate the final ppm value of the control and fortified samples according to the following equation:

$$(3)\quad \text{ppm CGA-152005} = \frac{\text{ng CGA-152005 found}}{\text{mg sample injected}}$$

Determine the recovery factor by first subtracting the background detector response, if any, in the control sample from the CGA-152005 response in the recovery sample. Calculate the recovery factor as a percentage (R%) by the equation:

$$(4)\quad R\% = \frac{\text{ppm CGA-152005 found}}{\text{ppm CGA-152005 added}} \times 100\%$$

III. RESULTS AND DISCUSSION

Recovery results for fortified control samples were used to calculate accuracy in terms of a mean, standard deviation (sd) and Coefficient of Variation (CV) for the limit of determination, and for all recovery results included in the validation.

The average recovery for samples fortified with CGA-152005 at the limit of determination of 0.01 ppm was 87% (sd: 15, CV: 17%, n=21) and 88% (sd: 13, CV: 15%, n=62) for all levels (See Table III).

Samples from two Metabolism studies[3,4] were analyzed during method validation. Corn from these studies was treated with either ^{14}C-phenyl-CGA-152005 or ^{14}C-triazine-CGA-152005 via stem-injection

(greenhouse grown plants) or a 40-g a.i./ha foliar spray (field grown plants).

Precision of the method was determined by calculating the mean, Coefficient of Variation and standard deviation of replicate analysis sets of each of the incurred ^{14}C-residue samples. Only some of the samples contained both enough plant material for triplicate analysis and ^{14}C levels high enough to quantitate by LSC and/or HPLC. The results are as follows: Phenyl-^{14}C-CGA-152005 injected corn foliage, mean = 0.031 ppm, sd: 0.003, CV: 9% (HPLC); phenyl-^{14}C-CGA-152005 injected corn stalk, mean = 0.007 triazine-^{14}C-CGA-152005 injected corn foliage, mean = 0.16, sd: 0.04, CV: 25% (HPLC). Overall, the precision of Analytical Method AG-590A is acceptable.

The extractability of the Analytical Method is determined by comparing the total ppm ^{14}C-residue found in the sample from combustion analysis to the ppm ^{14}C-residue found in the initial sample extract from Section II.C.2.0. The formula for the determination of % extractability is:

$$\% \text{ ext.} = \frac{\text{ppm } ^{14}\text{C-residue extract}}{\text{ppm } ^{14}\text{C-residue sample}} \times 100\%$$

The extractabilities for greenhouse grown stem-injected corn substrates were 69% and 102% for grain and foliage/stalk, respectively. The extractabilities for field grown spray-treated corn substrates were 95% and 42% for forages and fodder, respectively. Grain from field treated corn contained total incurred ^{14}C residues too low to quantitate.

The accountability of an Analytical Method is determined by comparing the total ppm ^{14}C-residue found in the sample, the ppm ^{14}C-residue found in the final fraction and the ppm analyte found in the final fraction to each other. The determinations of CGA-152005 by HPLC and of ^{14}C by LSC in the final fraction solutions correlated very well and showed that the cleanup procedures isolate CGA-152005 from any other metabolites or degradates. Also this Analytical Method was able to extract weathered residues from and determine parent compound in ^{14}C-CGA-152005 treated samples (Table IV and Figures 10-12).

This method has been validated under Protocol No. 106-91 with Analytical Method AG-590A as the final report. Results of this validation are shown in Tables III and IV and are reported in Residue Test Report RI-MV-003-91, No. 15.

IV. CONCLUSION

Analytical Method AG-590A is a valid and accurate method for the determination of parent residues of CGA-152005 in crops.

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-590A, are certified to be authentic accounts of the experiments.

R. E. M. Wurz, Research Scientist
Residue Chemistry
Biochemistry Department
910-632-2391

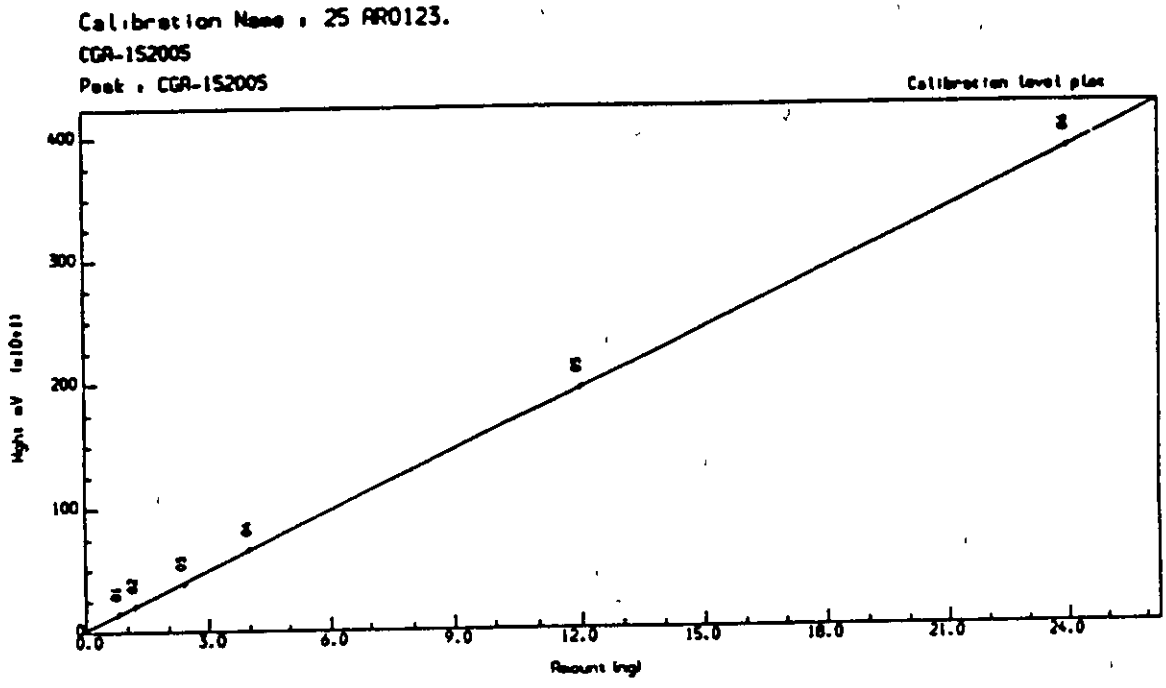
12/15/93
Date

TABLE I. LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS FOR DETERMINATION OF CGA-152005

Instrument:	Waters 501 HPLC pump (pump 2) or equivalent Perkin-Elmer Model Series-4 Solvent Delivery System (pump 1) or equivalent Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent ABI Spectroflow Model 783 Variable Wavelength UV Detector or equivalent Valco 6-port nitronic valve with electronic actuator or equivalent
Column Oven:	BioRad HPLC column heater, model number 125-0425 or equivalent
Oven Temp.:	30°C (both columns)
Column 1:	Brownlee Guard Cartridge, Sphere-5 cyanopropyl, 3 cm X 2.1 mm (Rainin cat. #CS-032) YMC 120A CN, 250 mm x 2.0 mm, 5-µm particle size (YMC Inc. cat. #MC-512)
Column 2:	Supelcosil LC-18-DB, 250mm x 2.1 mm, 5-µm particle size (Supelco cat. #5-7940M)
Mobile Phase 1:	3:7 ACN:0.1% H_3PO_4/water
Mobile Phase 2:	4:6 ACN:0.1% H_3PO_4/water
Retention Time:	~14 min. (Column 1) ~30 min (through both columns)
Detection:	ABI Kratos Spectroflow Model 783 Programmable Absorbance Detector or equivalent variable wavelength detector.
Wavelength:	225 nm
Attenuation:	0.006 AUFS
Flow Rate:	0.3-0.4 ml/min (both pumps)
Volume Injected:	40 µl
Run Time:	40 min/injection

TABLE II. TYPICAL STANDARDIZATION DATA FOR CGA-152005

Std. Wt. Inj. ng	Cal. Lev.	Peak Height µv
0.8000	01	152
1.2000	02	213
2.4000	03	390
4.0000	04	664
12.0000	05	1944
24.0000	06	3863



Constant : 1.85653E+1
1st degree : 1.60236E+2

Curve fit : Linear
Correlation coefficient : 0.99999
Standard error : 7.52789
Reported on 24-JAN-1992 at 10:06

TABLE III. SUMMARY OF RECOVERY DATA FOR CGA-152005

Sample Number	Corn Substrate	Fortification Level (ppm)	% Recovery
G.00A	Grain	0 (Control)	(<0.01 ppm)
G.01A, G.01B	Grain	0.01	63, 92
G.05A, G.05B	Grain	0.05	73, 76
G.00AR	Grain	0 (Control)	(<0.01 ppm)
G.01AR, G.01BR	Grain	0.01	120, 101
G.05AR, G.05BR	Grain	0.05	86, 69
G.00BR	Grain	0 (Control)	(<0.01 ppm)
G.10AR, G.10BR	Grain	0.10	106, 100
G.20AR, G.20BR	Grain	0.20	97, 98
GT.0C	Grain	0 (Control)	(<0.01 ppm)
GT.01	Grain	0.01	75
GT.02	Grain	0.05	84
FLP.0C, FLT.0C	0-Day Forage	0 (Control)	(<0.01 ppm) (<0.01 ppm)
FLP.10, FLT.10	0-Day Forage	0.1	95, 102
FLP2.0, FLT2.0	0-Day Forage	2.0	89, 97
FLP4.0, FLT4.0	0-Day Forage	4.0	102, 83
XFP.0C	Foliage	0 (Control)	(<0.01 ppm)
FP.01	Foliage	0.01	87
FP.20	Foliage	0.20	89
XFT.0C	Foliage	0 (Control)	(<0.01 ppm)
FT.02	Foliage	0.02	85
FT1.0	Foliage	1.0	83
F.00A	Forage	0 (Control)	(<0.01 ppm)
F.01A, F.01B	Forage	0.01	80, 83
F.05A, F.05B	Forage	0.05	92, 90
F.00B	Forage	0 (Control)	(<0.01 ppm)
F.10A, F.10B	Forage	0.10	73, 72
F.20A, F.20B	Forage	0.20	92, 60
FFP.0C	Forage	0 (Control)	(<0.01 ppm)
FFP.01	Forage	0.01	61
FFP.10	Forage	0.10	101
FFT.0C	Forage	0 (Control)	(<0.01 ppm)
FFT.01	Forage	0.01	110
FFT.05	Forage	0.05	94
FSP.0C, FST.0C	Silage Stage Forage	0 (Control)	(<0.01 ppm) (<0.01 ppm)
FSP.01, FST.01	Silage Stage Forage	0.01	102, 72
FSP.05, FST.05	Silage Stage Forage	0.05	83, 104
SP.0C	Stalk	0 (Control)	(<0.01 ppm)
SP.01	Stalk	0.01	77
SP.10	Stalk	0.20	91
ST.0C	Stalk	0 (Control)	(<0.01 ppm)
ST.01	Stalk	0.01	87
ST.20	Stalk	0.20	80
D.00A	Fodder	0 (Control)	(<0.01 ppm)

Mean = 88%, sd = 13, CV: 15%, n=62
*Samples analyzed but rejected due to documented problems during workup or analysis.

TABLE III. SUMMARY OF RECOVERY DATA FOR CGA-152005
(Continued)

Sample Number	Corn Substrate	Fortification Level (ppm)	% Recovery
D.01A, D.01B	Fodder	0.01	79, 103
D.05A, D.05B	Fodder	0.05	91, 96
D.00B	Fodder	0 (Control)	(<0.01 ppm)
D.10A, D.10B	Fodder	0.10	68, 99
D.20A, D.20B	Fodder	0.20)	72, 112
FDP.0C, FDT.0C	Fodder	0 (Control	(<0.01 ppm) (<0.01 ppm)
FDP.01, FDT.01	Fodder	0.01	78, 75
FDP.05, FDT.05	Fodder	0.05	72, Rej.*
OIL.0	Crude Oil	0 (Control)	(<0.01 ppm)
OIL.01A, OIL.01B	Crude Oil	0.01	Rej.*, 87
OIL.05A, OIL.05B	Crude Oil	0.05	84, 86
FLR.0	Flour	0 (Control)	(<0.01 ppm)
FLR.01A, FLR.01B	Flour	0.01	97, 92
FLR.05	Flour	0.05	102
FLR.10	Flour	0.10	85

Mean = 88%, sd = 13, CV: 15%, n=62
*Samples analyzed but rejected due to documented problems during workup or analysis.

TABLE IV. SUMMARY OF RESULTS FOR ^{14}C-CGA-152005 TREATED CORN

Sample ID	Study Number 54-91.1 Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
		(Sprayed Phenyl-^{14}C-CGA-152005)			
(0-Day Forage)					
FLP.SB	53434	3.44	1.63	94	1.61
(30-Day Forage)					
FFP.SA	53435	0.092	<0.01	97	0.002
FFP.SB	53435	"	<0.01	92	0.003
FFP.SC	53435	"	<0.01	96	0.002
(46-Day Silage Stage Forage)					
FSP.SA	53436	0.034	<0.01	112	<0.001
FSP.SB	53436	"	-NA-**	100	-NA-**
(93-Day Mature Fodder)					
FDP.SA	53437	0.048	<0.01	54	0.002
FDP.SB	53437	"	<0.01	52	0.001

Sample ID	Study Number 54-91.2 Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
		(Sprayed Triazine-^{14}C-CGA-152005)			
(0-Day Forage)					
FLT.SA	53405	3.30	1.69	100	1.30
(30-Day Forage)					
FFT.SA	53406	0.029	<0.01	79	0.001
FFT.SB	53406	"	<0.01	86	<0.001
(46-Day Silage Stage Forage)					
FST.SA	53407	0.048	<0.01	101	0.001
FST.SB	53407	"	<0.01	90	0.001
(93-Day Mature Fodder)					
FDT.SA	53408	0.009	<0.01	30	<0.001
FDT.SB	53408	"	<0.01	30	<0.001

* ^{14}C incurred levels determined by combustion/LSC by Metabolism Department. Reference Lab Notebooks 4002 and 4045.
** Sample results not available due to documented problems during workup or analysis.

COMMENTS: Results are corrected for procedural recoveries <100%.

TABLE IV. SUMMARY OF RESULTS FOR ^{14}C-CGA-152005 TREATED CORN (Continued)

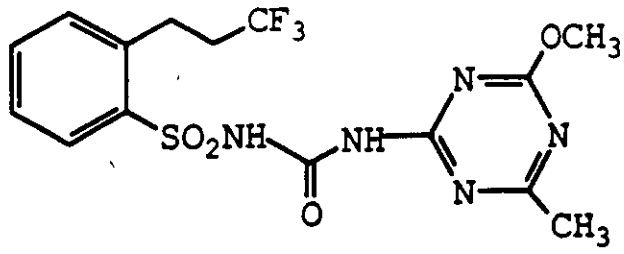
Sample ID	Study Number M91-168-007P Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
		(Injected Phenyl-^{14}C-CGA-152005)			
(Mature Foliage)					
XFP.IA	P91400161	0.308	0.032	99	0.032
XFP.IB	P91400161	0.308	0.028	104	0.030
XFP.IC	P91400161	0.308	0.033 (CV:9%)	96	0.031
(Mature Stalk)					
SP.IA	P91400078	0.195	<0.01	103	0.008
SP.IB	P91400078	0.195	-NA-**	108	0.007
SP.IC	P91400078	0.195	<0.01	108	0.006 (CV:14%)

Sample ID	Study Number M91-168-008P Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
		(Injected Triazine-^{14}C-CGA-152005)			
(Mature Foliage)					
XFT.IA	P91400175	1.28	0.14	87	0.15
XFT.IB	P91400175	1.28	0.14	90	0.15
XFT.IC	P91400175	1.28	0.21 (CV:25%)	94	0.19
(Mature Stalk)					
ST.IA	P91400061	0.262	-NA-**	103	-NA-**
ST.IB	P91400061	0.262	<0.01	134	0.006
ST.IC	P91400061	0.262	<0.01	99	0.006
(Mature Grain)					
GT.IA	P91400063	0.038	<0.01	70	<0.001
GT.IB	P91400063	0.038	<0.01	70	<0.001
GT.IC	P91400063	0.038	<0.01	68	<0.001

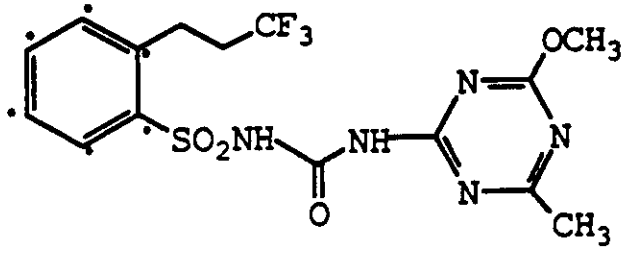
* ^{14}C incurred levels determined by combustion/LSC by Metabolism Department. Reference Lab Notebooks 3955 and 3921.
** Sample results not available due to documented problems during workup or analysis.

COMMENTS: Results are corrected for procedural recoveries <100%.

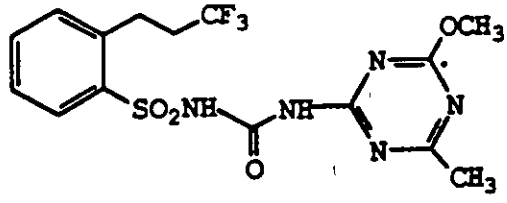
FIGURE 1. CHEMICAL NAME AND STRUCTURE



CGA-152005
N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-
2-(3,3,3-trifluoropropyl)-Benzenesulfonamide
CAS No. 94125-34-5



Phenyl Label CGA-152005
N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-
2-(3,3,3-trifluoropropyl)-[U-^{14}C]-Benzenesulfonamide



Triazine Label CGA-152005
N-[[(4-methoxy-6-methyl-1,3,5-[2-^{14}C]-triazin-
2-yl)amino]carbonyl]-2-(3,3,3-trifluoropropyl)-
Benzenesulfonamid

FIGURE 2. SCHEMATIC DIAGRAM OF THE HPLC COLUMN SWITCHING SYSTEM

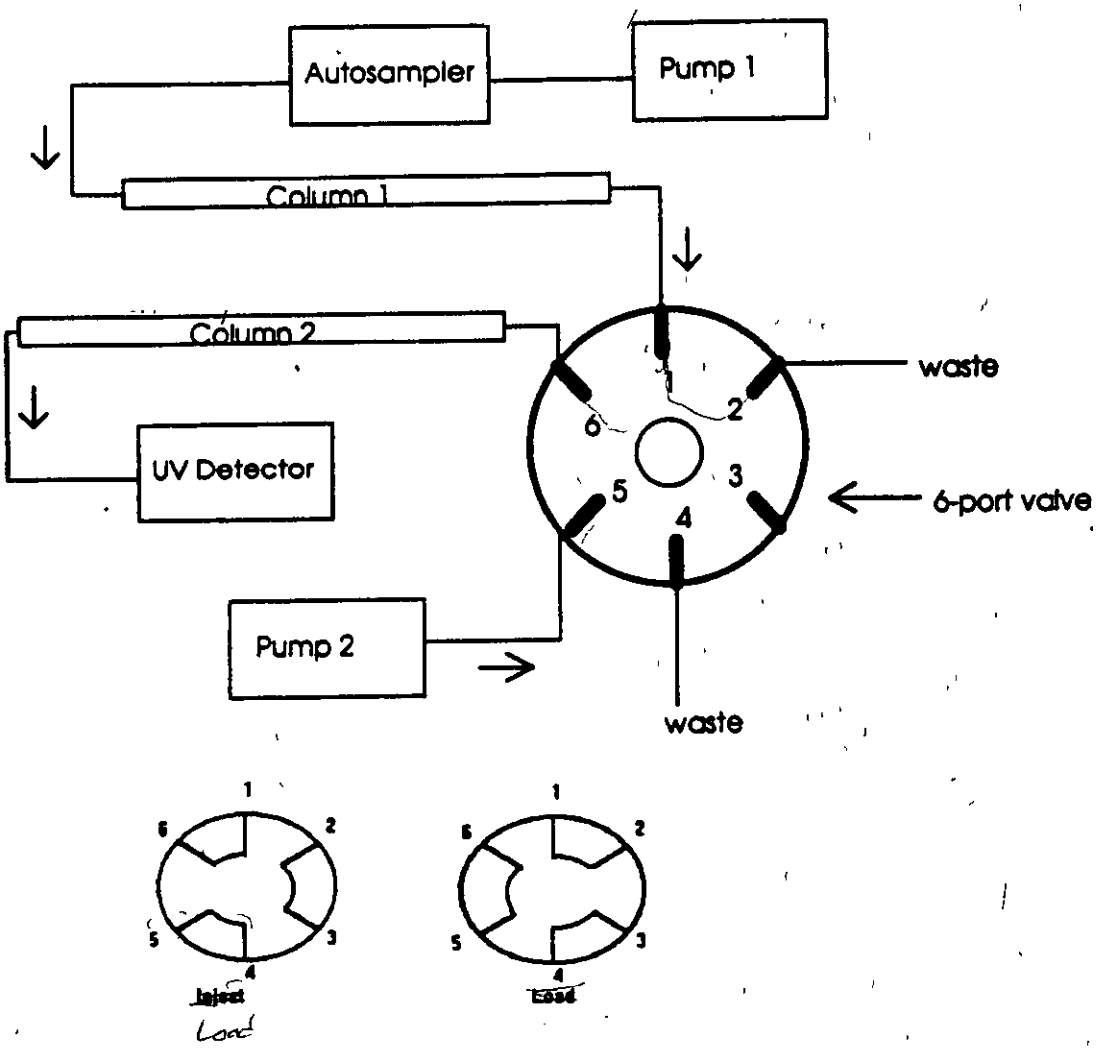


FIGURE 3. FLOW DIAGRAM FOR ANALYTICAL METHOD AG-590: SOLID SUBSTRATES

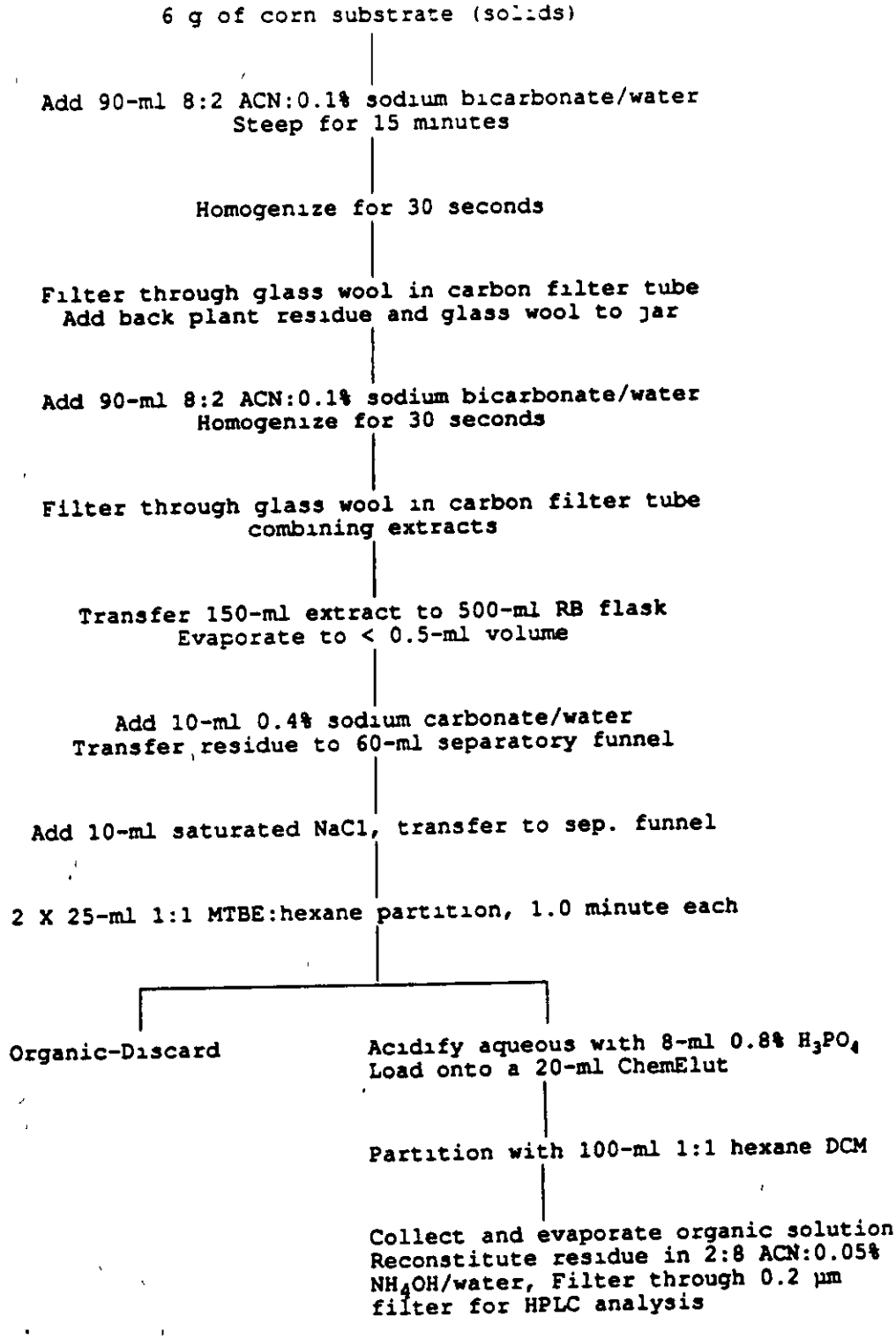


FIGURE 4. FLOW DIAGRAM FOR ANALYTICAL METHOD AG-590: OIL SUBSTRATES

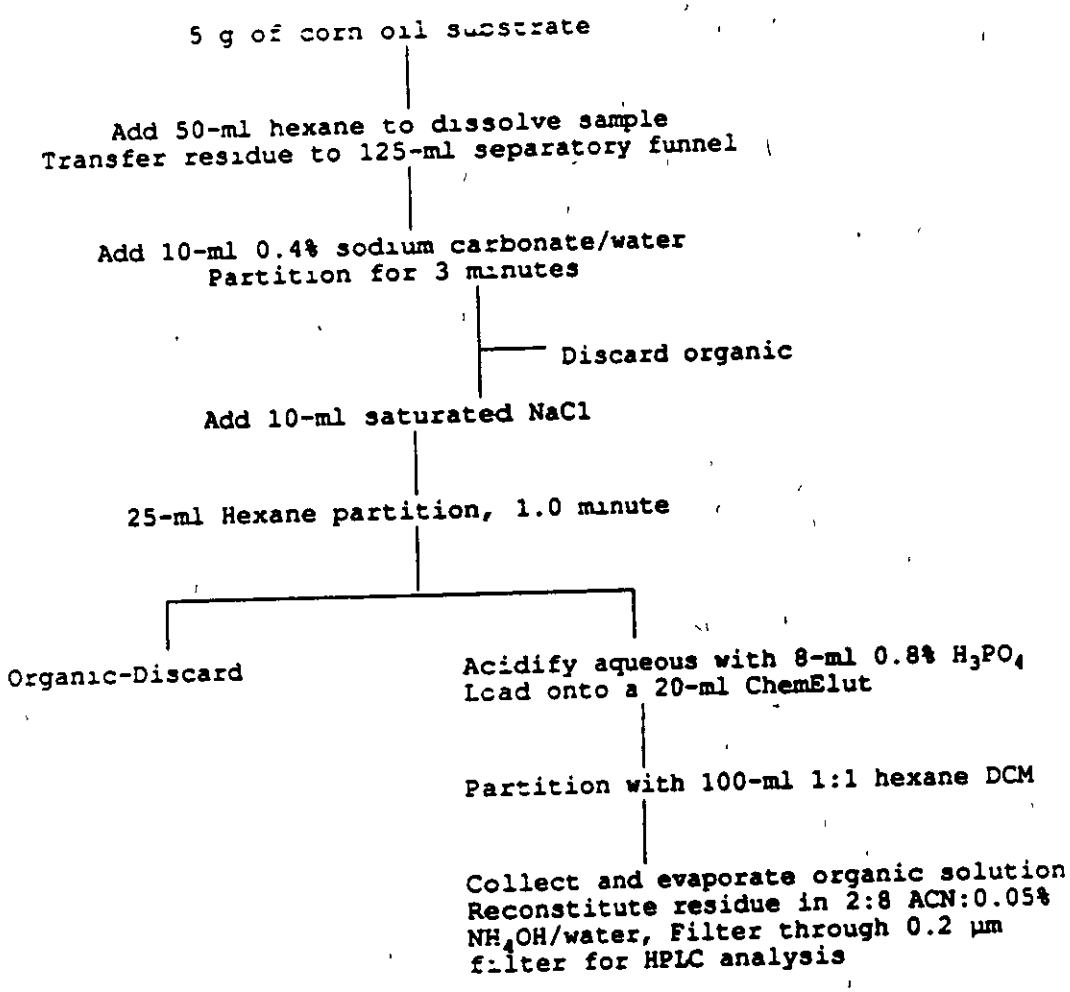
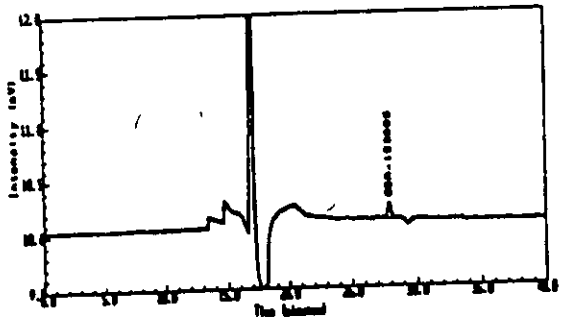
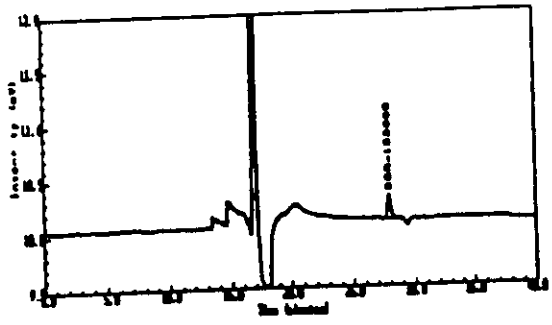


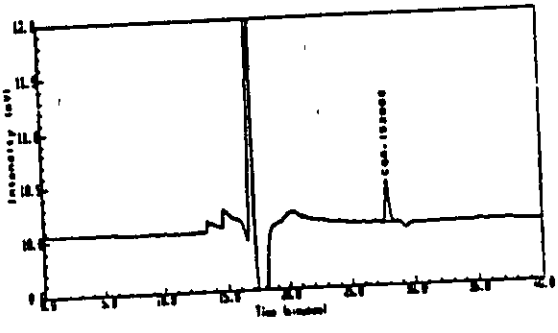
FIGURE 5. REPRESENTATIVE CHROMATOGRAMS FOR CGA-152005 STANDARDS



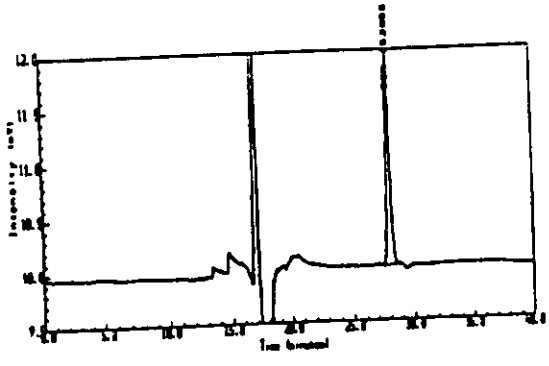
0.8 ng, CGA-152005 Standard



1.2 ng, CGA-152005 Standard

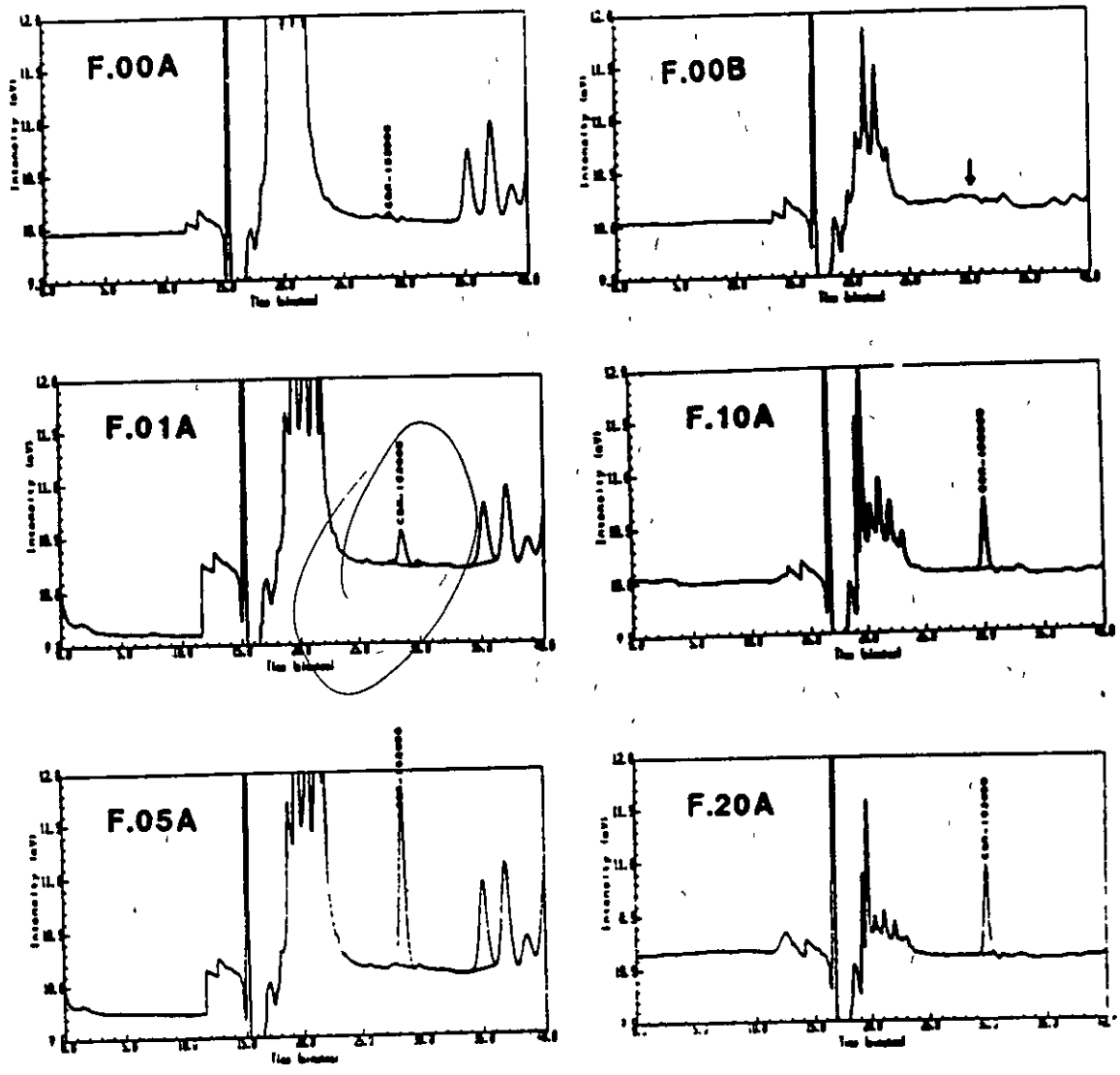


2.4 ng, CGA-152005 Standard



12 ng, CGA-152005 Standard

FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN FORAGE SAMPLES



F.00A: 0-day Corn Forage; 195 mg injected; 0.58 ng found; <0.01 ppm (0 003 ppm)

F 00B. 30-day Corn Forage; 195 mg injected; <0.8 ng found; <0.01 ppm

F.01A· 0-day Corn Forage + 0 01 ppm CGA-152005; 195 mg injected; 2 1 ng found; 0.011 ppm, 80% recovery

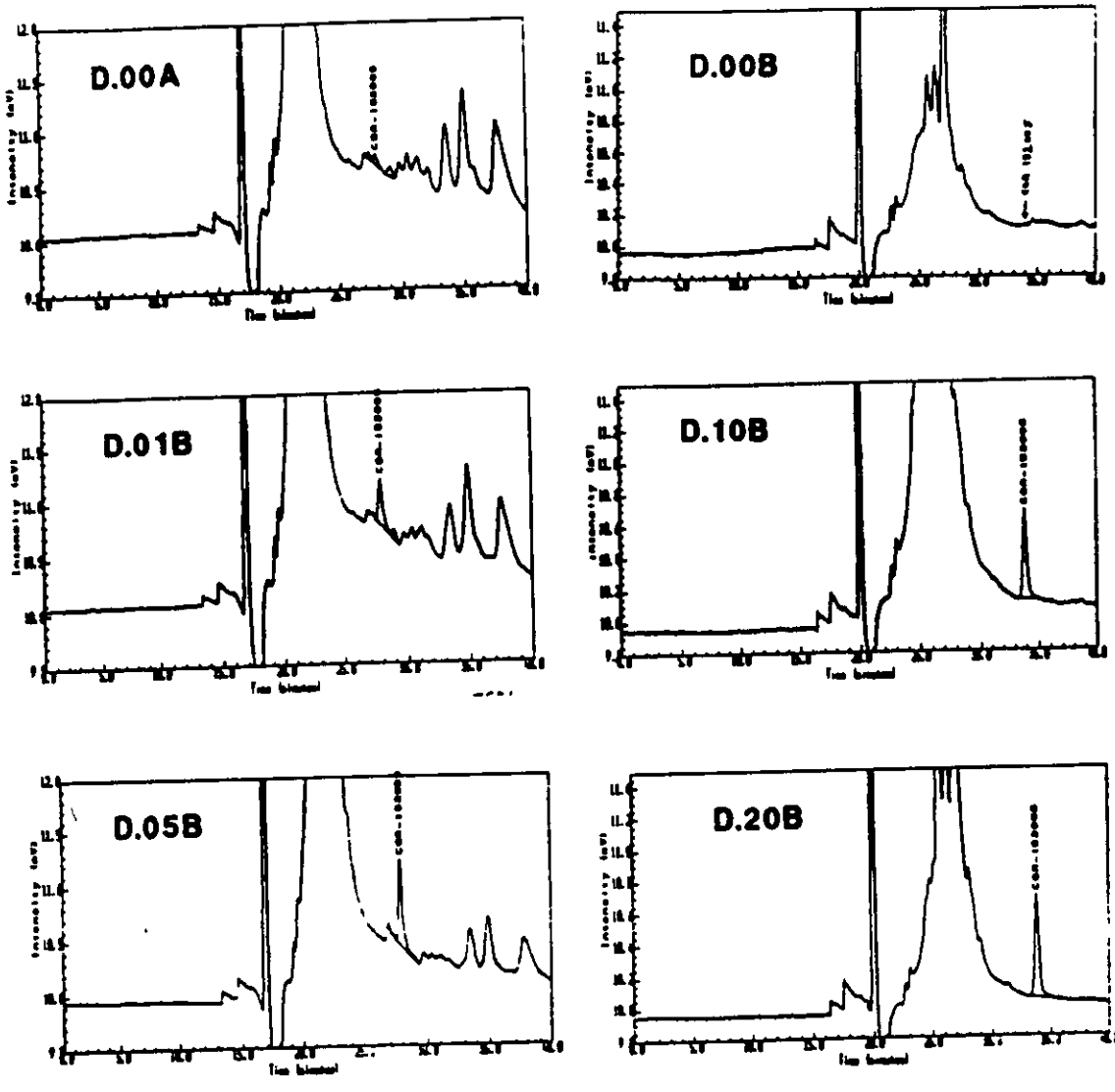
F.10A. 30-day Corn Forage + 0 10 ppm CGA-152005, 98 mg injected, 7.1 ng found; 0.073 ppm, 73% recovery

F 05A: 0-day Corn Forage + 0 05 ppm CGA-152005; 195 mg injected, 9.6 ng found; 0 049 ppm, 92% recovery

F.20A 30-day Corn Forage + 0 20 ppm CGA-152005, 49 mg injected; 8.9 ng found; 0 18 ppm, 92· recovery

(Recovery results corrected for control values)

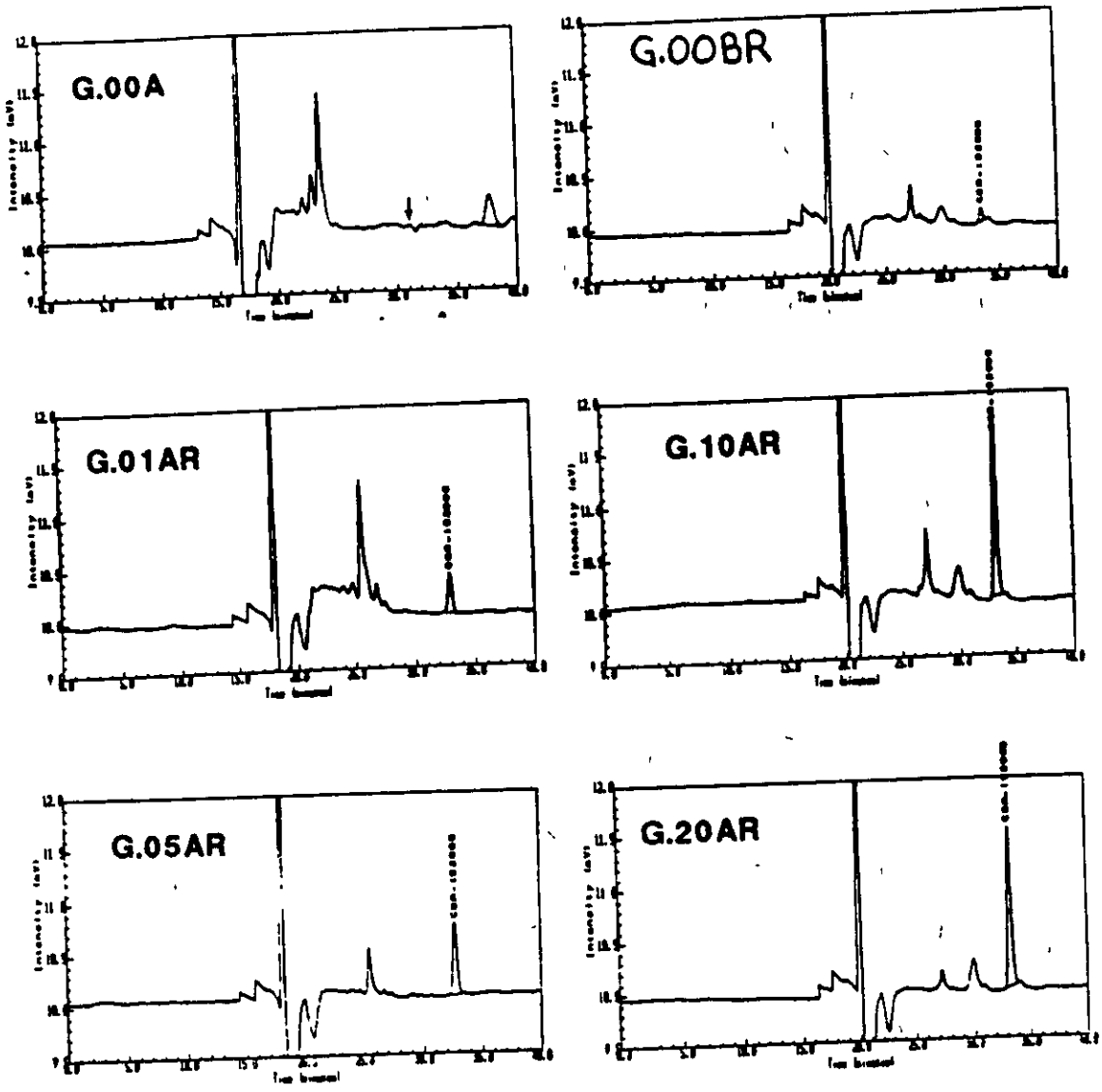
FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN FODDER SAMPLES



D 00A: Corn Fodder; 198 mg injected; 0.48 ng found, <0.01 ppm (0.002 ppm)
D.00B: Corn Fodder; 187 mg injected; <0 8 ng found, <0 01 ppm
D.01B: Corn Fodder + 0.01 ppm CGA-152005, 198 mg injected; 2 5 ng found, 0 013 ppm; 103% recovery
D 10B: Corn Fodder + 0.10 ppm CGA-152005; 90 mg injected; 8.9 ng found, 0.099 ppm; 99% recovery
D.05B: Corn Fodder + 0.05 ppm CGA-152005; 99 mg injected, 5 0 ng found, 0.050 ppm; 96% recovery
D 20B: Corn Fodder + 0 20 ppm CGA-152005, 45 mg injected, 10 ng found, 0 22 ppm; 112% recovery

(Recovery results corrected for control values)

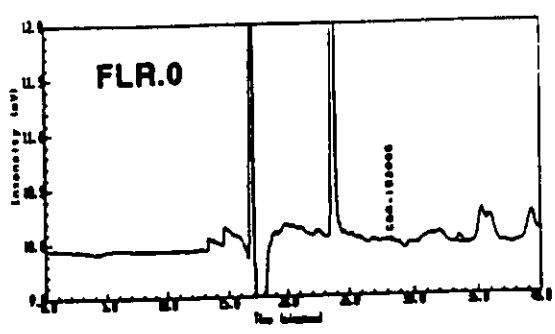
FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN GRAIN SAMPLES

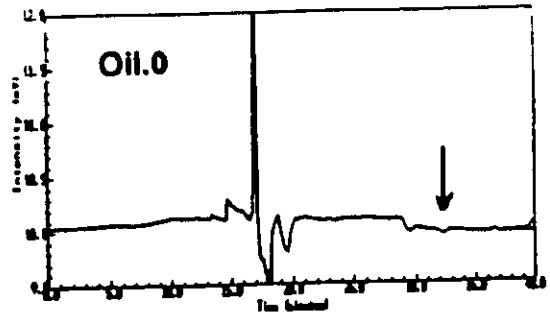


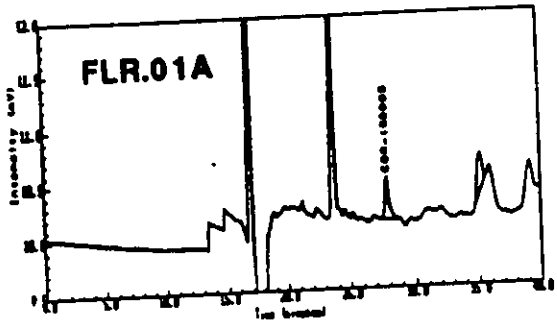
G 00A Corn Grain; 200 mg injected; <0.8 ng found; <0.01 ppm
G 00BR: Corn Grain, 200 mg injected; 0 72 ng found; <0 01 ppm (0.004 ppm)
G 01AR. Corn Grain + 0 01 ppm CGA-152005; 200 mg injected, 2.4 ng found; 0 012 ppm; 120% recovery
G 10AR Corn Grain + 0 10 ppm CGA-152005; 100 mg injected; 11 ng found, 0 11 ppm, 106% recovery
G 05AR· Corn Grain + 0 05 ppm CGA-152005, 100 mg injected; 4 3 ng found, 0 042 ppm, 86% recovery
G.20AR Corn Grain + 0 20 ppm CGA-152005, 50 mg injected, 9 9 ng found; 0 20 ppm, 97% recovery

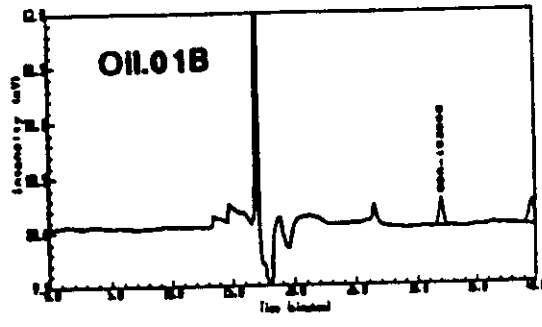
(Recovery results corrected for control values)

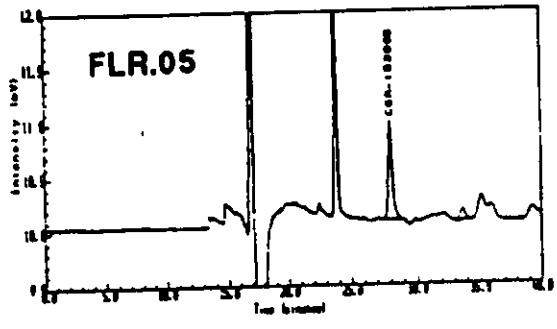
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FOR CONTROL AND FORTIFIED CONTROL CORN OIL AND FLOUR SAMPLES

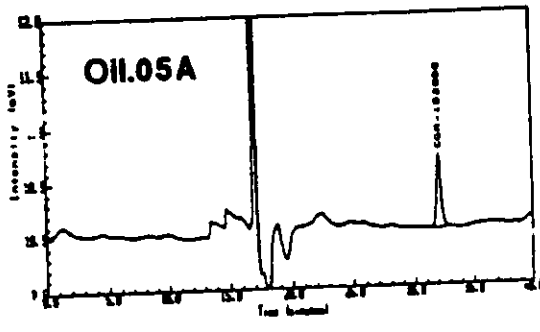








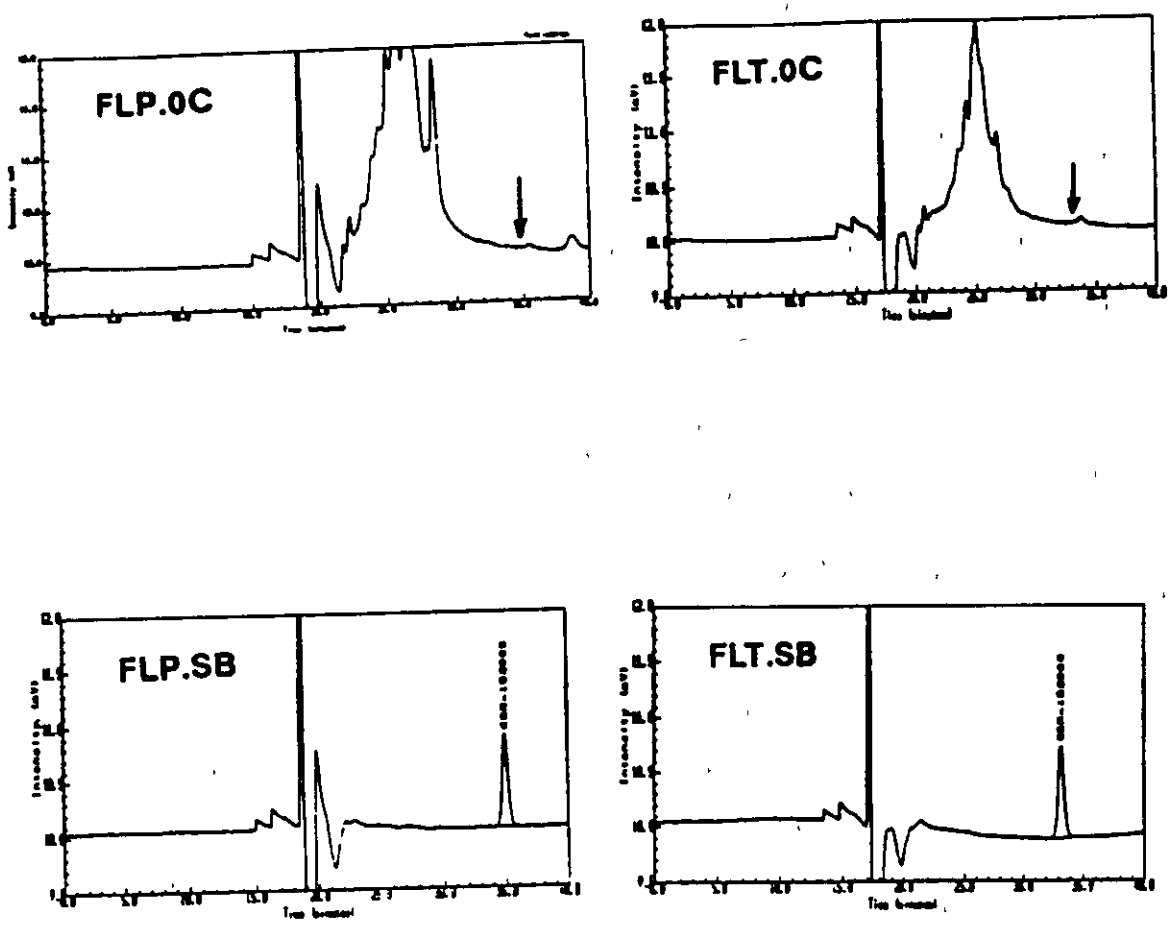




FLR.0: Corn Flour; 200 mg injected, <0.8 ng found; <0.01 ppm
Oil 0 Corn Crude Oil, 200 mg injected, <0.8 ng found; <0 01 ppm
FLR 01A: Corn Flour + 0.01 ppm CGA-152005, 200 mg injected, 2 1 ng found; 0 010 ppm, 97% recovery
Oil 01B: Corn Crude Oil + 0 01 ppm CGA-152005, 200 mg injected, 1.7 ng found, 0 009 ppm; 87% recovery
FLR 05 Corn Flour + 0 05 ppm CGA-152005, 100 mg injected, 5 2 ng found, 0.052 ppm, 102% recovery
OIL 05A Corn Crude Oil + 0 05 ppm CGA-152005; 100 mg injected, 4 2 ng found, 0 042 ppm; 84% recovery

(Recovery results corrected for control values)

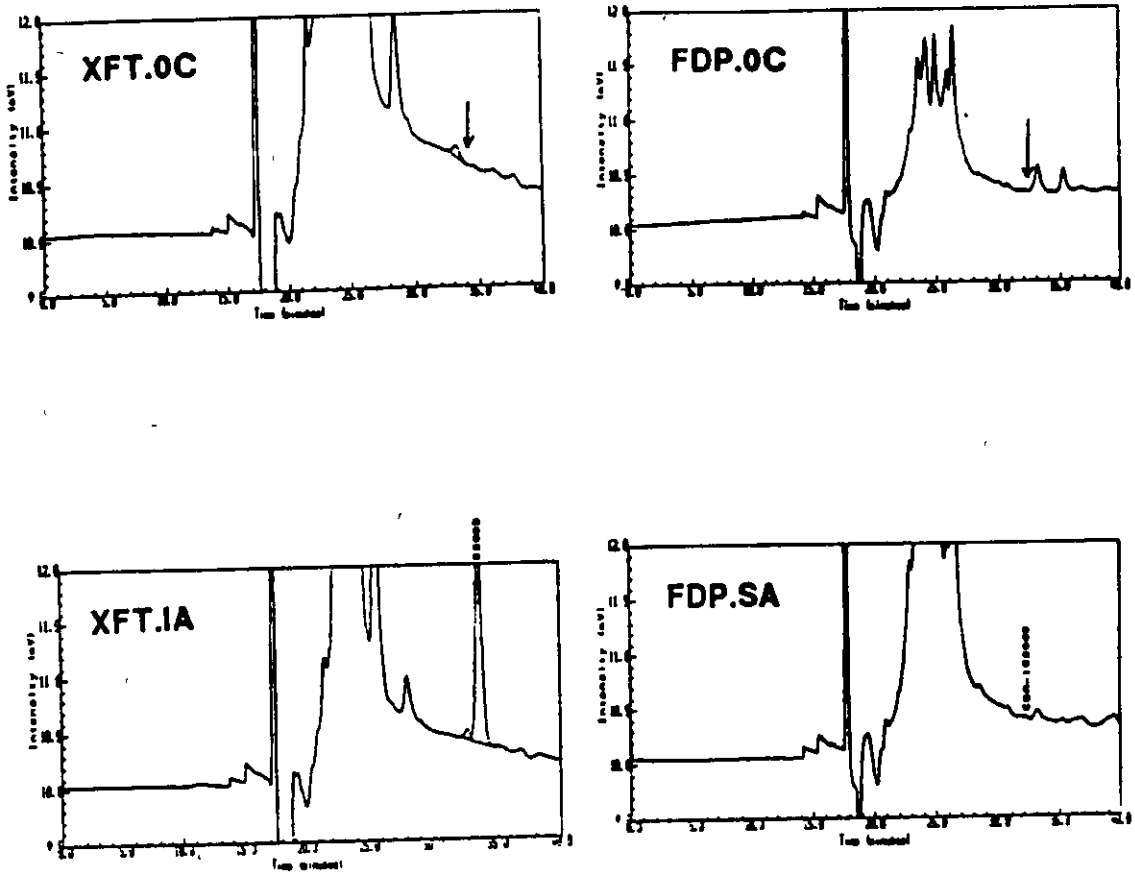
FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FOR ^{14}C-CGA-152005 TREATED CORN FORAGE SAMPLES



FLP.0C: 0-Day Corn Forage, 195 mg injected; <0.8 ng found; <0.01 ppm
FLT 0C: 0-Day Corn Forage; 195 mg injected; <0.8 ng found; <0.01 ppm
FLP.SB: 0-Day Corn Forage treated with phenyl-^{14}C-CGA-152005; 3 9 mg injected; 6 0 ng found; 1 63 ppm
FLT SB: 0-Day Corn Forage treated with triazine-^{14}C-CGA-152005; 3 9 mg injected; 6.2 ng found, 1.69 ppm

(Sample values corrected for procedural recoveries <100%)

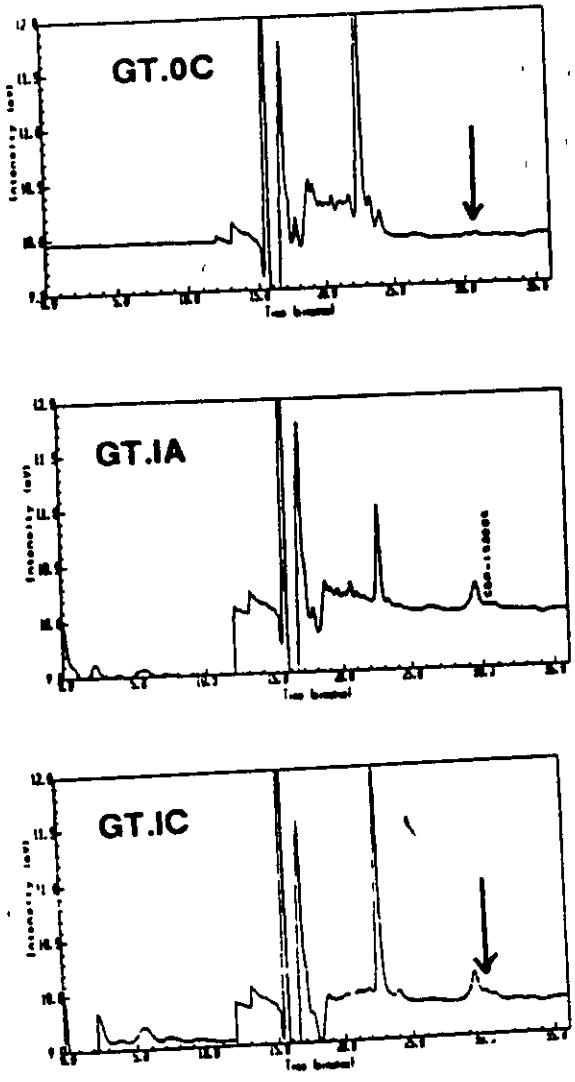
FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FOR ^{14}C-CGA-152005 TREATED CORN FODDER SAMPLES



XFT 0C. Corn Fodder (Foliage) 186 mg injected, <0 8 ng found; <0.01 ppm
FDP 0C: Corn Fodder 184 mg injected, <0 8 ng found, <0 01 ppm
XFT IA. Corn Fodder (Foliage) treated with injected triazine-^{14}C-CGA-152005. 96 mg injected, 11 ng found; 0 14 ppm
FDP SA Corn Fodder treated with phenyl-^{14}C-CGA-152005; 197 mg injected, 0 40 ng found, <0.01 ppm

(Sample values corrected for procedural recoveries <100%)

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FOR ^{14}C-CGA-152005 TREATED CORN GRAIN SAMPLES



GT 0C Corn Grain, 200 mg injected, <0 8 ng found, <0.01 ppm
GT IA. Corn Grain, treated with injected triazine-^{14}C-CGA-152005; 200 mg injected; <0 8 ng found, <0.01 ppm
GT IC. Corn Grain; treated with injected triazine-^{14}C-CGA-152005, 200 mg injected, <0 8 ng found; <0.01 ppm

(Sample values corrected for procedural recoveries <100%)

VII. REFERENCES

1. R. E. M. Wurz, Analytical Method AG-590, "Analytical Method for the Determination of CGA-152005 in Crops by High Performance Liquid Chromatography with Column Switching Including Validation Data."

2. Rolando Perez and Thomas Schreier, ADPEN Report #901-93-0108-002, "Independent Laboratory Confirmation of Ciba Analytical Method AG-590 ('Analytical Method for the Determination of CGA-152005 in Crops by High Performance Liquid Chromatography with Column Switching Including Validation Data']."

3. J. McFarland, ABR-93048; (Protocol 23-91) Study not completed, report in preparation.

4. J. McFarland, ABR-93047; (Protocol 54-91) Study not completed, report in preparation.

5. R. E. M. Wurz, Residue Test Report RI-MV-003-91 No. 1.

APPENDIX I

RESIDUE CHEMISTRY DEPARTMENT PROTOCOL NUMBER 106-91 AND AMENDMENTS 1 AND 2

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410

CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
RESIDUE CHEMISTRY DEPARTMENT
PROTOCOL NUMBER 106-91

VALIDATION OF "DRAFT" ANALYTICAL METHOD AG-590,"ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING INCLUDING VALIDATION DATA"

Study/Project No.:168982

STUDY DIRECTOR: R. E. M. Wurz

TITLE: Project Scientist

SIGNATURE:

DATE: 10/4/91

APPROVED BY: R.K. Williams

TITLE: Manager
Method Development

SIGNATURE:

DATE: 10/7/91

SPONSOR:
CIBA-GEIGY Corporation
Agricultural Division
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Quality Assurance Unit

Auditor:

Date: 10/4/91

TESTING FACILITY:

CIBA-GEIGY Corporation
Agricultural Division
Method Development Laboratory
Residue Chemistry Department
410 Swing Road
Greensboro, NC 27419

PROPOSED EXPERIMENTAL START DATE: October 15, 1991

PROPOSED EXPERIMENTAL TERMINATION DATE: November 30, 1991

PROPOSED STUDY COMPLETION DATE: December 15, 1991

STUDY OBJECTIVE

The objective of this study is to validate "Draft" Analytical Method AG-590 (Appendix I) for the quantitation of residues of CGA-152005 in corn substrates at a screening level of 0.01 ppm. This validation will be accomplished by analysis of control samples and fortified control samples to demonstrate the accuracy and precision, and samples from metabolism studies of corn treated with ^{14}C-CGA-152005 in order to determine the accountability, precision and total extractability of the method. Results of the determination of CGA-152005 in corn will be reported in Analytical Method AG-590 and Residue Test Report RI-MV-003-91.

TEST SUBSTANCES

CGA-152005: Lot #: S90-1490 (B06617), exp. date: 11/92, purity: 97.1%, Storage Condition - Room Temperature L-2066
Source: CIBA-GEIGY PTAS Department

Stock and standard solutions are stored refrigerated in L-2074.

TEST SYSTEM

Corn from the following sources will be analyzed and be referenced under Test No. RI-MV-003-91 (Inventory Numbers 13225.1, 13225.2 and 13225.3):

Corn control samples from Residue Chemistry Inventory Numbers 12059.5, 12059.7, 10549.4, 12033.1, 12035.1, 12033.2, 12035.2, 13063.2, 13218.2 and 11912.3.

Control and ^{14}C-treated corn samples from: Metabolism Department Protocol 23-91, Study Numbers M91-168-007P, and M91-168-008P (Stem injected phenyl and triazine ^{14}C labelled CGA-152005, Greenhouse grown). Metabolism Department Protocol 54-91, Experiment Numbers 54-91.1, 54-91.2 and 54-91.3 (Spray treated phenyl and triazine ^{14}C labelled CGA-152005, Field grown).

Sample code numbers are found in Tables I and II.

JUSTIFICATION OF TEST SYSTEM

Analysis of control and ^{14}C-CGA-152005 treated corn samples by "Draft" Analytical Method AG-590 (Appendix I) will be performed to determine the extractability of residues, accountability of the method, and precision of the method for CGA-152005. The accuracy and also precision of "Draft" Analytical Method AG-590 (Appendix I) will be demonstrated by analysis of control and fortified control corn samples for CGA-152005 in corn.

Analysis of corn treated with ^{14}C-CGA-152005 is required to determine the total extractability of "Draft" Analytical Method AG-590 (Appendix I) and to demonstrate the accountability of this method for its consideration as an EPA/FDA tolerance enforcement method.

The ^{14}C-CGA-152005 treated corn samples were selected to provide ppm levels of radioactive residue sufficient for quantitation, either by the method or by analysis of the radioactivity in the final fractions of the method. Corn samples treated with ^{14}C-CGA-152005 under typical field conditions were also selected for analysis even though the levels of radioactivity may be low, in order to evaluate the method's performance on treated field grown crops.

EXPERIMENTAL DESIGN

CIBA-GEIGY "Draft" Analytical Method AG-590 (Appendix I) will be used to determine CGA-152005.

Fortified Samples - "Draft" Analytical Method AG-590 (Appendix I).

^{14}C-Treated Samples - Biochemistry Standard Operating Procedure 4.67 rev. 1 (combustion analysis), Biochemistry Standard Operating Procedure 4.6 rev. 2 (liquid scintillation counting), and "Draft" Analytical Method AG-590 (Appendix I).

Modifications - Any modifications will be documented with protocol amendments.

The experiments will consist of the analysis of control and fortified control corn samples fortified at or above the screening level of "Draft" Analytical Method AG-590 (Appendix I), as well as samples that were treated with ^{14}C-CGA-152005. The preparation of standards and fortification of control samples will be performed according to procedures in "Draft" Analytical Method AG-590 (Appendix I).

The sets of samples to be analyzed in this study are outlined in Table I. The accuracy of the methods used in this study will be confirmed by the recovery results from the analyses of fortified control samples. The precision of the method will be determined by the reproducibility of the amounts of CGA-152005 determined by the method; and if the amounts of ^{14}C-CGA-152005 are below the screening level (0.01 ppm), replicate determinations of the total radioactivity in the final fractions will contribute to the determination of precision.

The amount of radioactivity (^{14}C) in the corn substrates has been determined from combustion analysis (Table II). CGA-152005 will be determined by high performance liquid chromatographic procedures of "Draft" Analytical Method AG-590 (Appendix I) and the radioactivity in the final fractions will be determined by liquid scintillation counting (SOP 4.6 rev. 2) and expressed as ppm values. The accountability of the method will be determined by the comparison of the total radioactivity combustion values with the analytical values determined by the method.

Total extractability of ^{14}C residues for the method will be determined by a comparison of the ^{14}C-CGA-152005 treated corn total radioactivity combustion values (Table II) with the radioactivity values determined by LSC from the sample extract solutions. The determination of total extractability measures the efficiency of the extraction procedure of the method and together with the total accountability is an indication of the method's ability to analyze weathered samples.

The control of bias in the study will be accomplished by the use of control samples for all fortification experiments. Other experimental design details are to be found in Appendix I, "Draft" Analytical Method AG-590.

RECORDS TO BE MAINTAINED

All personnel involved in this study will maintain laboratory notebooks or worksheets in which all data for the project will be recorded as required by good laboratory practice according to the procedures outlined in Metabolism and Residue Chemistry Standard Operating Procedure 8.1 rev. 4. Original chromatograms, computer printouts, etc., will be clearly labeled and kept in a separate file which will be clearly marked as Test Number RI-MV-003-91. All data placed in this file will be clearly labeled as to origin and referenced to the notebook and page of the corresponding work description. Raw data will be archived in the Metabolism and Residue Chemistry Archives under Residue Test Number RI-MV-003-91. Results of ^{14}C validation will be reported in Residue Chemistry Test Report format. Laboratory notebooks will remain in the possession of the analysts until the study is completed and then transferred to the Metabolism and Residue Department Archives. An Final Report in the form of Analytical Method AG-590 plus Residue Test Report RI-MV-003-91 will be issued for this study and will be archived in the Metabolism and Residue Chemistry Archives.

PROPOSED STATISTICAL METHODS

Statistical methods for regression analysis for a standard curve and quantitation of residues are described in "Draft" Analytical Method AG-590 (Appendix I).

Recovery results for fortified control samples will be used to calculate accuracy and precision in terms of a mean, standard deviation and Coefficient of Variation for the screening level, and for all recovery results included in the study.

Additional precision data will be determined by calculating the mean, range, standard deviation and Coefficient of Variation of replicate analyses of each of the ^{14}C incurred residue samples.

PERSONNEL

1. Study Director: Robert E. M. Wurz, Project Scientist.

2. Project Analysts: Robert E. M. Wurz, Project Scientist.
John Darnow, Senior Chemist
Marta Szolics, Associate Chemist.
Blanche King, Senior Lab. Technician.

TABLE I

RECOVERY SAMPLES TO BE ANALYZED

Residue Test No. RI-MV-003-91

Sample Number	Corn Substrate	Fortification Level (ppm)	Compound
G.00A	Grain	0 (Control)	---
G.01A	Grain	0.01	CGA-152005
G.01B	"	"	"
G.05A	Grain	0.05	CGA-152005
G.05B	"	"	"
G.00B	Grain	0 (Control)	---
G.10A	Grain	0.10	CGA-152005
G.10B	"	"	"
G.20A	Grain	0.20	CGA-152005
G.20B	Grain	"	"
F.00A	Forage	0 (Control)	---
F.01A	Forage	0.01	CGA-152005
F.01B	"	"	"
F.05A	Forage	0.05	CGA-152005
F.05B	"	"	"
F.00B	Forage	0 (Control)	---
F.10A	Forage	0.10	CGA-152005
F.10B	"	"	"
F.20A	Forage	0.20	CGA-152005
F.20B	Forage	0.20	CGA-152005
D.00A	Fodder	0 (Control)	---
D.01A	Fodder	0.01	CGA-152005
D.01B	"	"	"
D.05A	Fodder	0.05	CGA-152005
D.05B	"	"	"

TABLE I. (Continued)

Sample Number	Corn Substrate	Fortification Level (ppm)	Compound
D.00B	Fodder	0 (Control)	---
D.10A	Fodder	0.10	CGA-152005
D.10B	"	"	"
D.20A	Fodder	0.20	CGA-152005
D.20B	Fodder	0.20	CGA-152005
OIL.0	Oil	0 (Control)	CGA-152005
OIL.01A	Oil	0.01	CGA-152005
OIL.01B	Oil	0.01	CGA-152005
OIL.05	Oil	0.05	CGA-152005
OIL.10	Oil	0.10	CGA-152005
FLR.0	Flour	0 (Control)	CGA-152005
FLR.01A	Flour	0.01	CGA-152005
FLR.01B	Flour	0.01	CGA-152005
FLR.05	Flour	0.05	CGA-152005
FLR.10	Flour	0.10	CGA-152005

TABLE II.

SAMPLES FROM METABOLISM PROTOCOL NUMBER 23-91

Sample ID	Study Number M91-168-007P Code No.	Incurred ^{14}C level (ppm)*	CGA-152005 Fortification Level (ppm)	Corn Substrate	
FP.0C	P91400163C	0.003	CONTROL	Mature	Foliage
FP.01	P91400163C	"	0.01	"	"
FP.10	P91400163C	"	0.20	"	"
FP.IA	P91400161	0.308	---	"	"
FP.IB	P91400161	0.308	---	"	"
FP.IC	P91400161	0.308	---	"	"
SP.0C	P91400082C	0.003	CONTROL	Mature	Stalk
SP.01	P91400082C	"	0.01	"	"
SP.10	P91400082C	"	0.20	"	"
SP.IA	P91400078	0.172	---	"	"
SP.IB	P91400078	0.172	---	"	"
SP.IC	P91400078	0.172	---	"	"

Sample ID	Study Number M91-168-008P Code No.	Incurred ^{14}C level (ppm)*	CGA-152005 Fortification Level (ppm)	Corn Substrate	
FT.0C	P91400178C	0.006	CONTROL	Mature	Foliage
FT.05	P91400178C	"	0.05	"	"
FT.50	P91400178C	"	1.0	"	"
FT.IA	P91400175	1.28	---	"	"
FT.IB	P91400175	1.28	---	"	"
FT.IC	P91400175	1.28	---	"	"
ST.0C	P91400065C	0.003	CONTROL	Mature	Stalks
ST.01	P91400065C	0.003	0.01	"	"
ST.20	P91400065C	0.003	0.50	"	"
ST.IA	P91400061	0.411	---	"	"
ST.IB	P91400061	0.411	---	"	"
ST.IC	P91400061	0.411	---	"	"
GT.0C	P91400067C	0.003	CONTROL	Mature	Grain
GT.01	P91400067C	"	0.01	"	"
GT.02	P91400067C	"	0.05	"	"
GT.IA	P91400063	0.038	---	"	"
GT.IB	P91400063	0.038	---	"	"
GT.IC	P91400063	0.038	---	"	"

* Determined by combustion/LSC and converted to equivalents of CGA-152005.

TABLE II. (Continued)

SAMPLES FROM METABOLISM PROTOCOL NUMBER 54-91

Sample ID	Experiment No. 54-91.1 Code No.	Incurred ^{14}C level (ppm)	CGA-152005 Fortification Level (ppm)	Corn Substrate
FLP.0C	53391	TBD	CONTROL	0-Day Leaves
FLP.01	53391	"	0.01	" "
FLP.05	53391	"	0.05	" "
FLP.SA	53434	"	---	" "
FLP.SB	53434	"	---	" "
FLP.SC	53434	"	---	" "
FFP.0C	53392	TBD	CONTROL	30-Day Forage
FFP.01	53392	"	0.01	" "
FFP.05	53392	"	0.05	" "
FFP.SA	53435	"	---	" "
FFP.SB	53435	"	---	" "
FSP.0C	53393	TBD	CONTROL	Silage Stage Forage
FSP.01	53393	"	0.01	" " "
FSP.05	53393	"	0.05	" " "
FSP.SA	53436	"	---	" " "
FSP.SB	53436	"	---	" " "
FDP.0C	53394	TBD	CONTROL	Mature Fodder
FDP.01	53394	"	0.01	" "
FDP.05	53394	"	0.05	" "
FDP.SA	53437	"	---	" "
FDP.SB	53437	"	---	" "

Control substrates from Experiment 54-91.3

TABLE II. (Continued)

Sample ID	Experiment No. 54-91.2 Code No.	Incurred ^{14}C level (ppm)	CGA-152005 Fortification Level (ppm)	Corn Substrate
FLT.0C	53391	TBD	CONTROL	0-Day Leaves
FLT.01	53391	"	0.01	" "
FLT.05	53391	"	0.05	" "
FLT.SA	53405	"	---	" "
FLT.SB	53405	"	---	" "
FLT.SC	53405	"	---	" "
FFT.0C	53392	TBD	CONTROL	30-Day Forage
FFT.01	53392	"	0.01	" "
FFT.05	53392	"	0.05	" "
FFT.SA	53406	"	---	" "
FFT.SB	53406	"	---	" "
FST.0C	53393	TBD	CONTROL	Silage Stage Forage
FST.01	53393	"	0.01	" " "
FST.05	53393	"	0.05	" " "
FST.SA	53407	"	---	" " "
FST.SB	53407	"	---	" " "
FDT.0C	53394	TBD	CONTROL	Mature Fodder
FDT.01	53394	"	0.01	" "
FDT.05	53394	"	0.05	" "
FDT.SA	53408	"	---	" "
FDT.SB	53408	"	---	" "
FGP.0C	53396	TBD	CONTROL	Mature Grain
FGP.01	53396	"	0.01	" "
FGP.05	53396	"	0.05	" "
FGP.SA	53410	"	---	" "
FGP.SB	53410	"	---	" "

Control substrates from Experiment 54-91.3

RADIATION SAFETY COMMITTEE APPROVAL

PROTOCOL FOR VALIDATION OF "DRAFT" ANALYTICAL METHOD AG-590

Study/Project No. 168982

Corn samples treated with ^{14}C-CGA-152005 according to Metabolism Department Protocols 23-91 and 54-91 are approved for use in this study under Radioactive Materials Project RMP-2.

Signed: W.L. Secrest

W.L. Secrest
Radiation Safety Officer
Regulatory Affairs.

Date: 10/7/91

CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
RESIDUE CHEMISTRY DEPARTMENT
PO BOX 18300
410 SWING ROAD
GREENSBORO, NC 27419

--"DRAFT"--

ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING INCLUDING VALIDATION DATA

ANALYTICAL METHOD NO. AG-590

PROJECT NUMBER: 168982 PROTOCOL: 106-91

STUDY INITIATION DATE:

SUBMITTED BY:

Dr. R. E. M. Wurz Title: Project Scientist

Signature:

STUDY DIRECTOR:

Dr. R. E. M. WURZ Title: Project Scientist

Signature:

Completion Date:

APPROVED BY:

R. K. Williams Title: Manager
Method Development

Signature:

Date:

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I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for determination of residues of CGA-152005 in crops and crop fractions. The limit of detection of this method is 0.8 ng of CGA-152005 and the limit of determination is 0.01 ppm. The chemical name and structure of CGA-152005 is shown in Figure 1.

B. PRINCIPLE

A 6-g subsample of crop substrate is homogenized twice with fresh acetonitrile (ACN)/0.1% sodium bicarbonate aqueous solution. Both extracts are filtered through glass wool and combined. A 150-ml aliquot of extract is transfered to a flask and the volume reduced to <1 ml. Oil samples are dissolved in 50-ml hexane and extracted with 1:1 0.1% sodium carbonate:saturated sodium chloride solution and taken straight to the ChemElut. The concentrated extract is diluted with aqueous saturated sodium chloride solution and aqueous dilute sodium carbonate solution and partioned against methyl tert-butyl ether (MTBE)/hexane. The aqueous solution is retained and acidified with dilute phosphoric acid before being loaded onto a 20-ml Chem-Elut column. The sample on the Chem-Elut column is partitioned with 100-ml dichloromethane (DCM)/hexane and the organic solution is collected. The sample solution is evaporated to incipient dryness and the residue reconstituted in 20% ACN/0.05% ammonium hydroxide. Residue determination is done by narrow bore HPLC with column switching (250 X 2.0 mm Cyano column to a 250 X 2.1 mm Supelcosil LC-18-DB column) with UV detection at 225 nm.

II. MATERIALS AND METHODS

A. APPARATUS

1.0 Bottles, square amber wide mouth, 16 oz.
2.0 Bottles, Boston Round, narrow mouth, 8 oz.
3.0 Bottles, Nalgene, 250 ml.
4.0 Carbon filter tube
5.0 Concentration tube, minimum volume 25-ml
6.0 Disposable Pasteur pipets
7.0 Funnel, long stem, 12.5-cm. size
8.0 Funnel, powder, 80-mm.
9.0 Funnel, separatory, 60-ml & 125-ml with Teflon stopcock
10.0 Glass wool
11.0 Graduated cylinder, 50-ml, 100-ml or equivalent
12.0 Homogenizer, Polytron or equivalent
13.0 Round bottom flasks, 500-ml, 250-ml
14.0 Rotary evaporator, Buchii or equivalent
15.0 Vials, Wheaton, 2-ml. or equivalent
16.0 Volumetric pipets, 1-ml, 2-ml, 10-ml

B. REAGENTS

1.0 Acetonitrile (ACN), HPLC grade
2.0 Ammonium hydroxide (NH_4OH), ACS Reagent grade
3.0 Dichloromethane (DCM), HPLC grade
4.0 50% DCM/Hexane (v/v)
5.0 Hexane, HPLC grade (Fisher cat. #H302SK-4)
6.0 Methyl tert-butyl ether (MTBE), HPLC grade
7.0 Phosphoric acid (H_3PO_4), Certified ACS grade
8.0 Sodium chloride, Certified ACS grade
9.0 Saturated solution of sodium chloride in water
10. Sodium bicarbonate, Certified ACS grade
11. Sodium carbonate, Certified ACS grade
12. 0.1% Sodium carbonate/water (w/v)
13. 8:1 ACN:0.1% Sodium bicarbonate/water (w/v)
14. Water, HPLC grade
15. Chem Elut, 20-ml capacity (Varian cat. #1219-8008)
16. CGA-152005, Analytical Standard supplied by CIBA-GEIGY Corporation, 410 Swing Rd., Greensboro, NC 27419.

C. ANALYTICAL PROCEDURE

1.0 Sample Preparation

Samples are received and stored according to SOP 7.20. Samples are prepared under the general guidelines of the U.S.Food and Drug Administration Pesticide Analytical Manual Volume I, Section 141.

2.0 Extraction

2.1 Crop RAC's and Solid Fractions: Weigh a 6-g aliquot of crop substrate into an 8-oz square amber jar. Fortify with CGA-152005 at this point for recovery samples. Add 90-ml 8:1 ACN:0.1% sodium bicarbonate/water and let the sample steep for 15 minutes. Homogenize the sample with a Polytron homogenizer at medium power for 30 seconds. Filter the sample through a plug of glass wool at the apex of a carbon filter tube into a amber boston round bottle. Return any crop matrix in the carbon filter tube and the glass wool to the extraction jar. Rinse any matrix residue adhering to the carbon filter tube into the extraction jar with 90-ml 8:1 ACN:0.1% sodium bicarbonate/water.

2.2 Homogenize the sample plus glass wool and solvent again for 30 seconds and filter the extract through a new plug of glass wool at the apex of the carbon filter tube. Collect both extracts in the same bottle and refrigerate the sample extract if it is not to be used immediatly.

2.3 Oil Samples: Transfer 6 g of crude or refined oil to a flask and add 50-ml hexane to dissolve the sample. Transfer the organic solution to a 125-ml separatory funnel. Rinse the flask with precisely 10-ml 0.1% sodium carbonate solution then 10-ml saturated sodium chloride solution and add these rinses to the separatory funnel. Gently shake the

separatory funnel for 3 minutes then allow the phases to separate. Drain the lower aqueous phase back into the flask and carry this solution foreward to Section II.C.4.1. Discard the organic solution.

3.0 Partition Cleanup

3.1 Transfer a 150-ml aliquot of sample extract to a 500-ml round bottom flask and remove the solvent by rotary vacuum evaporation until the volume is <1 ml (bath temperature <40°C). Add 10-ml 0.1% sodium carbonate solution to the round bottom flask and sonicate to loosen or dissolve any adhering residue. Transfer the solution to a 60-ml separatory funnel.

3.2 Add 10-ml saturated sodium chloride solution to the 500-ml round bottom flask and swirl. Transfer the solution to the 60-ml separatory funnel in Section II.C.3.1. Add 25-ml 1:1 MTBE:hexane to the 500-ml round bottom flask and swirl. Also transfer the solution to the 60-ml separatory funnel above.

3.3 Stopper the 60-ml separatory funnel and shake for one minute taking care to vent the funnel. Allow the two layers to separate. Break any emulsion that may form and drain the lower, aqueous layer and any emulsion back into the 500-ml round bottom flask from Section II.C.3.2. Discard the upper organic layer and transfer the aqueous layer back to the separatory funnel.

3.4 Add 25-ml 1:1 MTBE:hexane to the 60-ml separatory funnel, stopper and shake for one minute. Break any emulsion that may form and drain the lower, aqueous layer and any remaining emulsion back into the 500-ml round bottom flask from Section II.C.3.3. Discard the upper organic layer.

4.0 Chem-Elut Cleanup

4.1 Add 8-ml 0.25% phosphoric acid solution to the aqueous layer in the 500-ml round bottom flask from Section II.C.3.4 (or the flask from Section II.C.2.3 for oil samples) and swirl. Transfer the sample solution to the 20-ml Chem-Elut by passing it through (rinsing) the 60-ml separatory funnel in which the partitions were done. Let the solution sit in the Chem Elut column for at least 5 minutes.

4.2 Attach a reservoir to the Chem-Elut and partition the sample with 100-ml 1:1 DCM:hexane. The flow through the Chem-Elut should be no greater than 2-3 ml per minute. The flow is controlled by attaching a nylon stopcock to the outlet of the column. Collect the organic solution in a 250-ml round bottom flask. Evaporate the solvent from the sample solution until the volume is approximately 10 ml (water bath <35°C). Quantitatively transfer the sample solution to a concentration tube using three 2-3-ml acetone washes. Evaporate the sample just to dryness and reconstitute in the appropriate volume of 20% ACN/0.05% ammonium hydroxide solution for analysis by HPLC.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

1.1 Install the HPLC system according to Table I and Figure 2. Control of the switching valve is accomplished via time-programmed contact closures of the detector.

1.2 Determine the retention time of CGA-152005 on Column #1 by connecting Column #1 directly to the detector and injecting 20 ng. of the analyte. (Inject 40 ul. of the 0.5 ng/ul standard solution prepared in Section II.I.1.0)

1.3 Reconect the system as shown in Figure 2. Program the valve to switch to the INJECT POSITION at the beginning of the CGA-152005 analyte peak and to return to the LOAD POSITION at the end of the analyte peak of CGA-152005.

1.4 Inject 20 ng. of CGA-152005 to determine its retention time through the two columns and to confirm that the valve time programming is correct.

2.0 Standardization

2.1 Calibrate the HPLC system with each analytical run by checking the retention time and detector response relative to previous runs. Retention times must not vary more than 2% and detector response must not vary more than 5% between runs.

2.2 Standardize the HPLC system by injecting 40-ul aliquots of standard solutions of CGA-152005 in a working range of 0.8-24 ng/injection. Generate a linear regression from the data by comparing detector response and ng injected.

E. INTERFERENCES

None

F. CONFIRMITORY TECHNIQUES

None.

G. TIME REQUIRED

A skilled analyst can complete the extraction and analysis of a set of 6-8 samples in 8 working hours.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Some samples may develop emulsions after shaking (Section II.C.2.2 and II.C.3.3). These may be cleared if allowed to settle out slightly and then gently stirred with a glass rod.

2.0 During the evaporation of samples solutions in Sections II.C.3.1 and II.C.4.2 any water bath used must not have a temperature >35°C and the samples should be removed as soon as they are ready. Excessive temperature, especially when the sample has gone to dryness, leads to analyte decomposition.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Standard CGA-152005 Solutions

1.1 Weigh 10 mg of CGA-152005 analytical standardinto a 100-ml volumetric flask and dilute to the mark with ACN.

1.2 Make serial dilutions of the 0.1 mg/ml standard solution with 20% ACN/0.05% ammonium hydroxide solution (w/v) to give a series of fortification/analytical standards in a range of 0.02 ug/ml to 3.0 ug/ml of CGA-152005. Store the standard solutions in amber bottles at 4°C in the dark when not in use.

J. DETERMINATION OF SAMPLE RESIDUES

1.0 Inject 40-ul aliquots of sample extracts from Section II.C. into the HPLC under the same conditions as for standards. Make appropriate dilutions of the samples in 2:8 ACN:0.05% ammonium hydroxide/water solution to bring the sample peak heights within the range of the standard curve. Compare the peak heights of the unknown samples to the standard curve or enter the peak height into a least squares program to determine the nanograms of CGA-152005 in the injected aliquot. Typical chromatograms for control and procedural recovery samples are shown in Figures 5 and 6.

2.0 Calculate the residue results in terms of ppm of CGA-152005 by using the following equation:

$$(1) \quad ppm = \frac{(ng\ CGA\text{-}152005\ Found)\ (100)}{(mg\ sample\ injected)\ (R\%)}$$

Where mg sample injected is calculated as follows: (Equation 2)

$$(2) \quad mg\ inj. = \frac{(G)\ (V_a)(V_i)}{(V_e)\ \ (V_f)}$$

G = milligrams sample extracted

V_a = aliquot volume

Ve = extraction volume

V_i = injection volume (ul)

V_f = total volume of final injection solution (ul)

R% = recovery ratio given by equation 4

3.0 Fortification Experiments

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified immediately prior to extraction with CGA-152005.

3.1 Add 1.0 ml of a 0.06 ug/ml standard solution of CGA-152005 to 6 g. of control crop prior to the addition of extraction solvent for a 0.01 ppm fortification. Use correspondingly larger amounts of standards (volume should not exceed 2 ml) for higher fortifications. Analyze control and freshly fortified samples along with the treated samples according to the procedures of the method.

3.2 Calculate the final ppm value of the control and fortified samples according to the following equation:

$$(3) \quad ppm\ CGA\text{-}152005 = \frac{ng\ CGA\text{-}152005\ found}{g\ sample\ injected}$$

Determine the recovery factor by first subtracting the background detector response, if any, in the control sample from the CGA-152005 response in the recovery sample. Calculate the recovery factor as a percentage (R) by the equation:

$$(4) \quad R\% = \frac{\text{ppm CGA-152005 found}}{\text{ppm CGA-152005 added}} \times 100\%$$

III. RESULTS AND DISCUSSION

This method has been validated under Protocol No. 121-90 and used for the analysis of control, CGA-152005 fortified control and ^{14}C-CGA-152005 treated corn samples. The objective of Protocol 106-91 was to validate "Draft" Analytical Method AG-590 for the quantitation of residues of CGA-152005 in crops at a screening level of 0.01 ppm. Results of these analyses are shown in Table III and are reported in Residue Test Report RI-MV-003-91, No. 1.

Test substance ID, test system ID, protocol amendments, protocol deviations and circumstances affecting quality and integrity of data are also reported in Residue Test Report RI-MV-003-91, No. 1. All raw data associated with this study, retained samples and the original final report and protocol are archived in the Metabolism and Residue Chemistry Archives or freezer storage facility at CIBA-GEIGY Corporation, Greensboro, NC.

IV. CONCLUSION

Analytical Method AG-590 is a valid and accurate method for the determination of parent residues of CGA-152005 in crops.

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-590, are certified to be authentic accounts of the experiments.

Robert K. Williams, Manager — Date
Method Development
Residue Chemistry Department
919-632-2295

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-590 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

Robert E.M. Wurz, Ph.D. — Date
Study Director

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TABLE I.

LIQUID CHROMATOGRAPHIC OPERATING CONDITIONS FOR DETERMINATION OF CGA-152005

Instrument:	Waters 501 HPLC pump (pump 2) or equivalent Perkin-Elmer Model Series-4 Solvent Delivery System (pump 1) or equivalent Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent ABI Spectroflow Model 783 Variable Wavelength UV Detector
Column Oven:	BioRad HPLC column heater, model number 125-0425
Oven Temp.:	30°C (both columns)
Column 1:	Brownlee Guard Cartridge, Spheri-5 cyanopropyl, 3 cm X 2.1 mm (Rainin cat. #CS-032) Spherisorb CN, 250 mm x 2.0 mm, 5 um particle size (Phase Separations cat. #830925) or YMC 120A CN, 250 mm x 2.0 mm, 5 um particle size (YMC Inc. cat. #MC-512)
Column 2:	Supelcosil LC-18-DB, 250mm x 2.1 mm, 5 um particle size (Supelco cat. #5-7940M)
Mobile Phase 1:	3:7 ACN:0.1% H_3PO_4/water
Mobile Phase 2:	4:6 ACN:0.1% H_3PO_4/water
Retention Time:	~14 min. (Column 1) ~30 min (through both columns)
Detection:	ABI Kratos Spectroflow Model 783 Programmable Absorbance Detector or equivalent variable wavelength detector.
Wavelength:	225 nm
Attenuation:	0.005 AUFS
Flow Rate:	0.3 ml/min (both pumps)
Volume Injected:	40 ul
Chart Speed:	0.25 cm/min
Run Time:	40 min/injection

TABLE II.

TYPICAL STANDARDIZATION DATA FOR CGA-152005

TABLE III.

SUMMARY OF RECOVERY DATA FOR CGA-152005

FIGURE 1.

CHEMICAL NAME AND STRUCTURE

CGA-152005

1-(4-Methoxy-6-methyl-triazin2-yl)-3-[2-(3,3,3-trifluoropropyl)-phenylsulfonyl]-urea

FIGURE 2.

SCHEMATIC DIAGRAM OF THE HPLC COLUMN SWITCHING SYSTEM

FIGURE 3.

FLOW DIAGRAM FOR ANALYTICAL METHOD AG-590

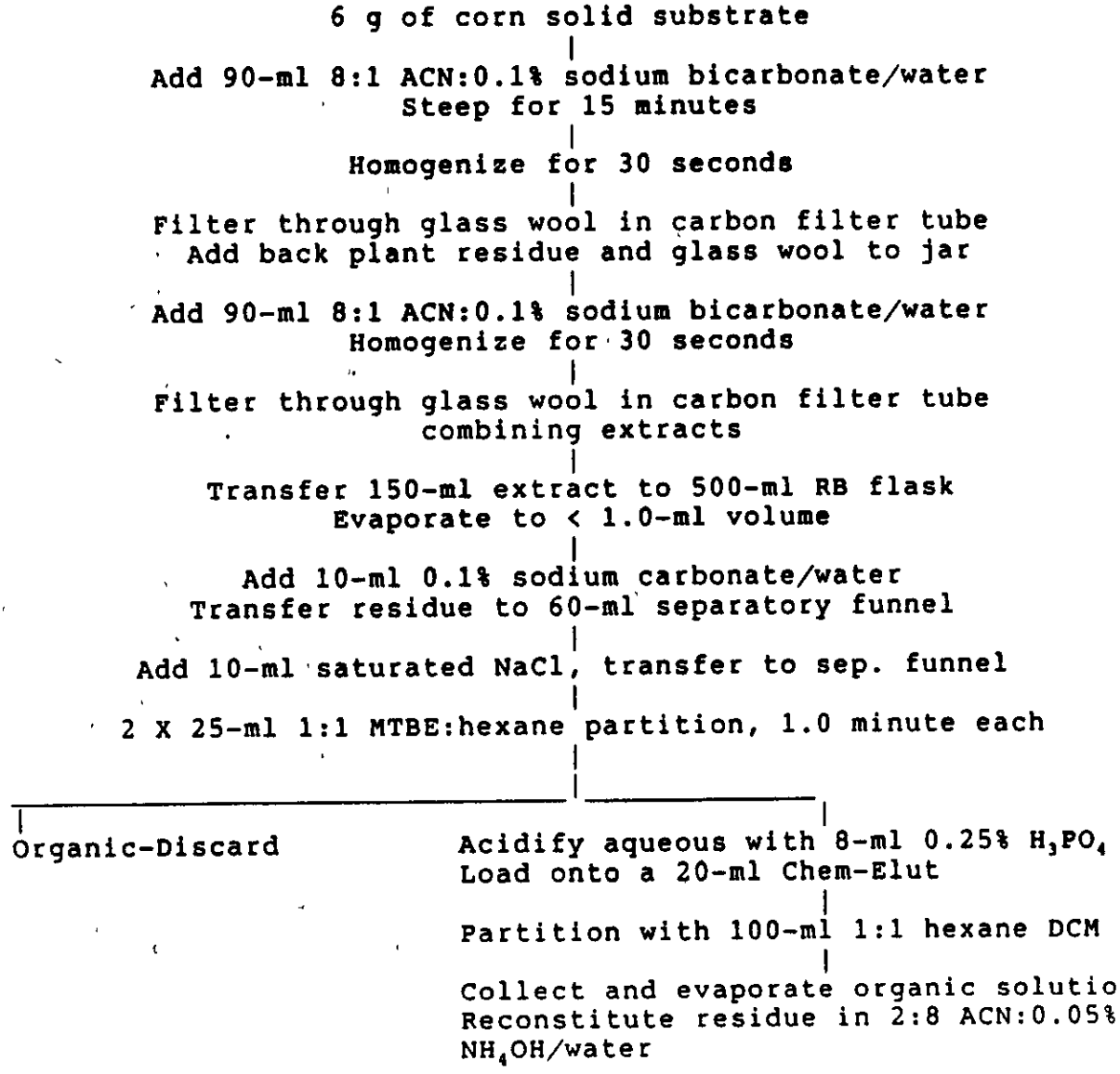

FIGURE 4.

Representative Chromatograms for CGA-152005 Standards

RESIDUE CHEMISTRY PROTOCOL AMENDMENT

AMENDMENT LIST NUMBER: 1

PROTOCOL NUMBER: 106-91

TITLE: VALIDATION OF "DRAFT" ANALYTICAL METHOD AG-590, "ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING DATA INCLUDING VALIDATION DATA"

PROJECT NUMBER: 168982

EFFECTIVE DATE: 10/22/91

AMENDMENTS:

1. Addition of samples: The samples below were added for analysis.

SAMPLES FROM METABOLISM PROTOCOL NUMBER 23-91

Sample ID	Study Number M91-168-007P Code No.(ppm)*	Incurred ^{14}C level Level (ppm)	CGA-152005 Fortification	Corn Substrate
XFP.0C	P91400163C	0.003	CONTROL	Mature Foliage
FP.05	P91400163C	"	0.05	" "
FP.50	P91400163C	"	1.0	" "
FP.20	P91400163C	"	0.20	" "
XFP.IA	P91400161	0.308	---	" "
XFP.IB	P91400161	0.308	---	" "
XFP.IC	P91400161	0.308	---	" "

Sample ID	Study Number M91-168-008P Code No.(ppm)*	Incurred ^{14}C level Level (ppm)	CGA-152005 Fortification	Corn Substrate
XFT.0C	P91400178C	0.006	CONTROL	Mature Foliage
FT.02	P91400178C	"	0.02	" "
FT1.0	P91400178C	"	1.0	" "
XFT.IA	P91400175	1.28	---	" "
XFT.IB	P91400175	1.28	---	" "
XFT.IC	P91400175	1.28	---	" "

2. Change in analytical procedure: In Section II.C.3.4 of "Draft" Analytical Method AG-590, 0.1% sodium carbonate solution is replaced by 0.4% sodium carbonate solution. In Section II.C.4.1 of "Draft" Analytical Method AG-590, 0.25% phosphoric acid solution is replaced by 0.8% phosphoric acid solution.

3. Extraction solvent: In Section II.C.2.1 and Figure 3, 8:1 ACN:0.1% sodium bicarbonate/water should be 8:2 ACN:0.1% sodium bicarbonate/water.

4. Table II. changes: The following changes have been made to Table II.

SAMPLES FROM METABOLISM PROTOCOL NUMBER 54-91

Sample ID	Experiment No. 54-91.1 Code No.	Incurred ^{14}C level (ppm)	CGA-152005 Fortification Level (ppm)	Corn Substrate
FLP.0C	**	---	CONTROL	0-Day Forage
FLP.10	**	---	0.10	" "
FLP2.0	**	---	2.0	" "
FLP4.0	**	---	4.0	" "
FLP.SB	53434	3.44	---	0-Day Leaves
**: Residue Test #RI-MV-003-91, ID. #56607, Inv. #13225.3, Control.				
FFP.0C	53392	0.003	CONTROL	30-Day Forage
FFP.01	53392	"	0.01	" "
FFP.10	53392	"	0.10	" "
FFP.SA	53435	0.092	---	" "
FFP.SB	53435	"	---	" "
FFP.SC	53435	"	---	" "
FSP.0C	53393	0.002	CONTROL	Silage Stage Forage
FSP.01	53393	"	0.01	" " "
FSP.05	53393	"	0.05	" " "
FSP.SA	53436	0.034	---	" " "
FSP.SB	53436	"	---	" " "

Control substrates from Experiment 54-91.3

Sample ID	Experiment No. 54-91.2 Code No.	Incurred ^{14}C level (ppm)	CGA-152005 Fortification Level (ppm)	Corn Substrate
FLT.0C	**	---	CONTROL	0-Day Forage
FLT.10	**	---	0.10	" "
FLT2.0	**	---	2.0	" "
FLT4.0	**	---	4.0	" "
FLT.SB	53405	3.30	---	0-Day Leaves
**: Residue Test #RI-MV-003-91, ID. #56607, Inv. #13225.3, Control.				
FFT.0C	53392	0.003	CONTROL	30-Day Forage
FFT.01	53392	"	0.01	" "
FFT.05	53392	"	0.05	" "
FFT.SA	53406	0.029	---	" "
FFT.SB	53406	"	---	" "
FST.0C	53393	0.002	CONTROL	Silage Stage Forage
FST.01	53393	"	0.01	" " "
FST.05	53393	"	0.05	" " "
FST.SA	53407	0.048	---	" " "
FST.SB	53407	"	---	" " "

Control substrates from Experiment 54-91.3

REASON(S):

1. These samples were analyzed either because the first two sets of analyses were not acceptable due to poor recoveries or because a recovery fortification level was changed.

2. The changes in solution strength were necessary to handle unusually acidic samples.

3. Typographical error.

4. Combustion data for these samples became available after the study was initiated. Incurred and fortified levels are updated. Sample amounts were determined and 0-day samples were very small so the number analyzed was reduced while another control sample was substituted for the 0-day control.

CHANGE INITIATED BY: _____ DATE: 11/14/91
CURRENT STUDY DIRECTOR

CHANGE AUTHORIZED BY: —N/A— DATE: _____
(IF APPLICABLE) MANAGEMENT SIGNATURE

AMENDMENTS TO BE DISTRIBUTED PER PROTOCOL DISTRIBUTION LIST

RESIDUE CHEMISTRY PROTOCOL AMENDMENT

AMENDMENT LIST NUMBER: 2

PROTOCOL NUMBER: 106-91

TITLE: VALIDATION OF "DRAFT" ANALYTICAL METHOD AG-590, "ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING DATA INCLUDING VALIDATION DATA"

PROJECT NUMBER: 168982

EFFECTIVE DATE: 12/12/91

AMENDMENTS:

1. Sample information: Information is updated for the samples below.

SAMPLES FROM METABOLISM PROTOCOL NUMBER 23-91

Sample ID	Experiment No. 54-91.1 Code No.	Incurred ^{14}C level (ppm)	CGA-152005 Fortification Level (ppm)	Corn Substrate
FDP.0C	53394	0.003	CONTROL	Mature Fodder
FDP.01	53394	"	0.01	" "
FDP.05	53394	"	0.05	" "
FDP.SA	53437	0.048	---	" "
FDP.SB	53437	"	---	" "

Control substrates from Experiment 54-91.3

Sample ID	Experiment No. 54-91.2 Code No.	Incurred ^{14}C level (ppm)	CGA-152005 Fortification Level (ppm)	Corn Substrate
FDT.0C	53394	0.003	CONTROL	Mature Fodder
FDT.01	53394	"	0.01	" "
FDT.05	53394	"	0.05	" "
FDT.SA	53408	0.009	---	" "
FDT.SB	53408	"	---	" "

Control substrates from Experiment 54-91.3

2. Sample deletion: The samples below will not be analyzed in this study:

FGP.0C	53396	0.003	CONTROL	Mature	Grain
FGP.01	53396	"	0.01	"	"
FGP.05	53396	"	0.05	"	"
FGP.SA	53410	"	---	"	"
FGP.SB	53410	"	---	"	"

3. Addition of samples: The samples below were added for analysis.

Sample Number	Corn Substrate	Fortification Level (ppm)	Compound
G.00AR	Grain	0 (Control)	---
G.01AR	Grain	0.01	CGA-152005
G.01BR	"	"	"
G.05AR	Grain	0.05	CGA-152005
G.05BR	"	"	"
G.00BR	Grain	0 (Control)	---
G.10AR	Grain	0.10	CGA-152005
G.10BR	"	"	"
G.20AR	Grain	0.20	CGA-152005
G.20BR	Grain	"	"

4. Sample set change: The oil sample set will consist of crude oil and have two replicates of the 0.05 ppm level.

Sample Number	Corn Substrate	Fortification Level (CGA-152005)
OIL.0	Crude Oil	0 (Control)
OIL.01A	Crude Oil	0.01
OIL.01B	Crude Oil	0.01
OIL.05A	Crude Oil	0.05
OIL.05B	Crude Oil	0.05

5. Change in Procedure: The extraction procedure under Section II.C.2.3 in the draft method was replaced with the following.

2.3 Oil Samples: Transfer 5 g of crude or refined oil to a 125-ml flask and add 50-ml hexane to dissolve the sample. Transfer the organic solution to a 125-ml separatory funnel. Rinse the flask with precisely 10-ml 0.4% sodium carbonate solution and add this rinse to the separatory funnel. Gently shake the separatory funnel for 3 minutes then allow the phases to separate (Caution: emulsions form easily). Drain the lower aqueous phase and any remaining emulsion back into the flask and discard the upper, organic layer. Discard the organic solution.

2.4 Add 10-ml saturated sodium chloride solution to the aqueous solution in the flask and transfer the combined volumes back into the separtory funnel. Add 25-ml hexane to the searatory funnel and shake for one minute. Allow the layers to separate, then drain the lower aqueous layer into the 125-ml flask and carry this solution on to Section II.C.4.1. Discard the organic layer.

6. Additional Study Personnel: Added to the list of study personnel is Amy Riley, Laboratory Technician.

REASONS:

1. Combustion data for these samples became available after the study was initiated. Incurred and fortified levels are updated. The incurred ^{14}C value for Metabolism sample #53437 is the average of 3 sets of combustions (reference LNB's #4002 & #4045).

2. The incurred residues for this grain set is so low that they will be undetectable and therefore these samples are not suitable for experiments meant to determine extractability and accountability.

3. These sets were added to give more information on the performance of the method with grain samples.

4. Corn crude oil was chosen to represent the worst case oil sample that this method could analyze. The change of fortification levels was made to give a better estimate of precision of the method with this substrate.

5. The extraction procedure for oil was changed because the original procedure had been developed for refined oils and was not adequate for crude oils. The replacement procedure results in acceptable recoveries for crude oil samples.

6. Additional qualified laboratory personnel were available to work on the project and become familiar with the method.

CHANGE INITIATED BY: _____ DATE: 1/27/92
CURRENT STUDY DIRECTOR

CHANGE AUTHORIZED BY: N/A DATE: _____
(IF APPLICABLE) MANAGEMENT SIGNATURE

AMENDMENTS TO BE DISTRIBUTED PER PROTOCOL DISTRIBUTION LIST

APPENDIX II

SEPARATE DOCUMENTS ACCOMPANYING THIS REPORT

1. R.E.M. Wurz, Analytical Method AG-590, "Analytical Method for the Determination of Chromatography with Column Switching Including Validation Data Column Switching Including Validation Data."

2. Rolando Perez and Thomas Schreier, ADPEN Report #901-93-0108-002, Independent Laboratory Confirmation of Ciba Analytical Method AG-590 ('Analytical Method for the Determination of CGA-152005 in Crops by High Performance Liquid Chromatography with Column Switching Including Validation Data')."

3. ABR-93048 (formerly ABR-93001), Protocol No. 23-91, "Uptake and Metabolism of CGA-152005 in Greenhouse Grown Corn after Spray Treatment or Stem Injection with Phenyl-14C-CGA-152005 and Triazine-14C-CGA-152005." Ruhi Rezaaiyan, Ph.D. (Note: Study Director replacement recorded in Protocol No. 23-91, Amendment 4, July 2, 1993.)

4. ABR-93047 (formerly ABR-93002), Protocol No. 54-91, "Uptake and Metabolism of CGA-152005 in Field Grown Corn after Spray Treatment with Phenyl-14C-CGA-152005 and Triazine-14C-CGA-152005." Ruhi Rezaaiyan, Ph.D. (Note: Study Director replacement recorded in Protocol No. 54-91, Amendment No. 3, July 2, 1993.)

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APPENDIX III

RESIDUE TEST REPORT RI-MV-003-91 REPORT NO. 1

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RESIDUE CHEMISTRY DEPARTMENT
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
GREENSBORO, NORTH CAROLINA

RESIDUE TEST REPORT

RESIDUE TEST NUMBER: RI-MV-003-91

REPORT NO.: 1

PROJECT NUMBER: 168982
PROTOCOL NUMBER: 106-91 and Amendments #1, #2
TEST SUBSTANCE: CGA-152005
TEST SYSTEM: Corn Grain, Fodder, Forages, Oil and Flour
LOCATION: N/A NO. OF ANALYSES: 116
LABORATORY: CIBA-GEIGY Method Development

DESCRIPTION: Control and fortified control corn samples and corn samples treated with phenyl or triazine labelled ^{14}C-CGA-152005 from metabolism studies were analyzed for residues of CGA-152005 by Analytical Method AG-590 in order to validate the method. Radioactive extractability and accountability determinations were also made on incurred ^{14}C-CGA-152005 from treated corn samples. Control corn grain, fodder, forages, oil and flour samples were fortified with CGA-152005 to generate recovery data for method validation.

SUBMITTED BY: R. E. M. Wurz, Research Scientist

STUDY DIRECTOR: R. E. M. Wurz, Research Scientist

SIGNATURE: [signature]

DATE: 3/20/92

APPROVED BY: R. K. Williams

TITLE: Manager
Method Development

SIGNATURE: [signature]

APPROVAL DATE: 3/20/92

DISTRIBUTION: R. A. Kahrs
B. J. King
A. Riley
M Szolics
R. K. Williams
R. E. M. Wurz
File

RESIDUE TEST REPORT

FIELD TEST NUMBER. RI-MV-003-91 PROTOCOL NUMBER. 106-91

REPORT NUMBER 1 PROJECT NUMBER: 168982

BIOLOGY SECTION

Corn substrates from the following sources were referenced under Test No. RI-MV-003-91 (Inventory Numbers 13225 1, 13225.2 and 13225.3):

Corn control samples from Residue Chemistry Inventory Numbers 12059.5, 12059.7, 10549.4, 12033.1, 12035 1, 12035 1, 12033 2, 12035 2, 13063.2, 13218.2 and 11912.3.

Control and ^{14}C-treated corn samples from: Metabolism Department Protocol 23-91, Study Numbers M91-168-007P (Greenhouse injected Phenyl-^{14}C-CGA-152005) and M91-168-008P (Greenhouse injected Triazine-^{14}C-CGA-152005). Metabolism Department Protocol 54-91, Experiment Numbers 54-91 1 (Field sprayed Phenyl-^{14}C-CGA-152005), 54-91.2 (Field sprayed Triazine-^{14}C-CGA-152005), 54-91.3 (Field Controls). Field corn samples from Experiment Numbers 54-91.1 and 54-91 2 were sprayed with 40 g ai/ha.

CIRCUMSTANCES AFFECTING QUALITY OR INTEGRITY OF DATA

None

DEVIATIONS FROM PROTOCOL

After reconstitution of samples in 2·8 ACN.0.05% NH_4OH water (Section II.C.4.2), the sample final solutions were filtered through a 0 2 µm filter into the injection vials in order to remove particulate material.

J. Darnow did not participate in this study.

0 1% Sodium Carbonate is used in Section II C 3 1 (Typographical error).

Mature corn stalk samples from injected corn greenhouse studies were recombusted to better determine the incurred ^{14}C residue Sample P91400061 had 0.262 ppm, sample 53437 had 0.048 ppm, and sample P91400078 had 0.195 ppm incurred ^{14}C. These values supersede those in the protocol.

TEST AND REFERENCE SUBSTANCES

Analytical Standard	Identification No.	Specific Activity	Purity	Reanalysis Date
CGA-152005	S90-1490	--	97.1%	11/92

SAMPLE IDENTIFICATION NUMBERS

Each analyzed corn sample was given a specific sample number as recorded in Protocol 106-91, and Lab Notebook No. 4127

STUDY PERSONNEL

M Szolics, Associate Chemist (MS)
R E M Wurz, Research Scientist (REMW)
A L Riley, Laboratory Technician (ALR)
B J King, Senior Laboratory Technician (BJK)

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-003-91 PROTOCOL NUMBER 106-91

REPORT NUMBER: 1 PROJECT NUMBER. 168982

ANALYTICAL SECTION

METHODOLOGY

METHOD NUMBER	COMMENTS
AG-590	^{14}C incurred residue analysis and fortified controls for recovery data of CGA-152005 for Method Validation.

ANALYSES

DATE EXTRACTED	DATE ANALYZED	NO. OF ANALYSES	LABORATORY	ANALYST(S)
(Forages) 10/22,24/91 11/6,11,12,14,19/91 12/4,11/91	10/24,25/91 11/8,12,14,15,19,20/91 12/9,13/91	53	CIBA-GEIGY Method Development	REMW, BJK
(Fodders/Stalk) 10/29/91, 11/1/91 12/30/91, 1/6,8,20/92	10/31/91, 11/1/91 12/31/91, 1/7,10,23/92	32	CIBA-GEIGY Method Development	REMW, MS, BJK, ALR
(Grain) 11/5/91 12/11,17/91 1/8/92	11/7/91 12/12,19/91 1/10/92	21	CIBA-GEIGY Method Development	REMW, MS, BJK
(Crude Oil) 1/17/92	1/17/92	5	CIBA-GEIGY Method Development	REMW
(Flour) 1/20/92	1/21/92	5	CIBA-GEIGY Method Development	REMW,ALR

SUMMARY

^{14}C-CGA-152005 treated, control and fortified control corn samples were analyzed by Analytical Method AG-590 for the determination of CGA-152005. The limit of detection is 0 8 ng of CGA-152005 and the limit of determination is 0 01 ppm for all substrates

The average recovery for all control corn samples fortified at the limit of determination is 87% (sd = 15, CV = 17%, n = 21). The average recovery for all levels in all fortified samples is 88% (sd = 13, CV = 15%, n = 62).

Metabolism samples were treated either with ^{14}C-phenyl- or ^{14}C-triazine-CGA-152005 by field spray or greenhouse injection. The average extractability of total radioactivity for all field grown corn with spray application was 95%, and 42% for forages and fodder respectively The average extractability of total radioactivity for all greenhouse grown corn stem-injected with ^{14}C-CGA-152005 was 102% and 69% for foliage/stalk and grain respectively

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-003-91 PROTOCOL NUMBER 106-91

REPORT NUMBER 1 PROJECT NUMBER: 168982

ANALYTICAL SECTION (Continued)

RESULTS

SUMMARY OF METHOD VALIDATION DATA FOR METHOD AG-590

FORTIFIED CORN CONTROL SAMPLE RECOVERY DATA

Sample Number	Corn Substrate	Fortification Level (ppm)	% Recovery
G 00A	Grain	0 (Control)	(<0.01 ppm)
G 01A, G 01B	Grain	0 01	63, 92
G 05A, G.05B	Grain	0.05	73, 76
G 00AR	Grain	0 (Control)	(<0.01 ppm)
G.01AR, G 01BR	Grain	0 01	120, 101
G 05AR, G.05BR	Grain	0.05	86, 69
G 00BR	Grain	0 (Control)	(<0.01 ppm)
G.10AR, G 10BR	Grain	0.10	106, 100
G.20AR, G 20BR	Grain	0.20	97, 98
GT 0C	Grain	CONTROL	(<0.01 ppm)
GT 01	Grain	0.01	75
GT 02	Grain	0 05	84
FLP.0C	0-Day Forage	CONTROL	(<0.01 ppm)
FLP 10	0-Day Forage	0 1	95
FLP2 0	0-Day Forage	2.0	89
FLP4 0	0-Day Forage	4 0	102
FLT 0C	0-Day Forage	CONTROL	(<0.01 ppm)
FLT 10	0-Day Forage	0.10	102
FLT2.0	0-Day Forage	2 0	97
FLT4 0	0-Day Forage	4.0	83
XFP 0C	Foliage	CONTROL	(<0 01 ppm)
FP 01	Foliage	0 01	87
FP 20	Foliage	0 20	89
XFT 0C	Foliage	CONTROL	(<0 01 ppm)
FT 02	Foliage	0 02	85
FT1 0	Foliage	1.0	83
F 00A	Forage	0 (Control)	(<0 01 ppm)
F 01A, F 01B	Forage	0 01	80, 83
F 05A, F 05B	Forage	0.05	92, 90
F 00B	Forage	0 (Control)	(<0 01 ppm)
F 10A, F 10B	Forage	0.10	73, 72
F 20A, F 20B	Forage	0 20	92, 60
FFP.0C	Forage	CONTROL	(<0 01 ppm)
FFP.01	Forage	0 01	61
FFP 10	Forage	0 10	101
FFT 0C	Forage	CONTROL	(<0 01 ppm)
FFT 01	Forage	0 01	110
FFT 05	Forage	0 05	94
FSP 0C	Silage Stage Forage	CONTROL	(<0.01 ppm)
FSP.01	Silage Stage Forage	0.01	102
FSP 05	Silage Stage Forage	0 05	83
FST 0C	Silage Stage Forage	CONTROL	(<0 01 ppm)
FST 01	Silage Stage Forage	0 01	72
FST 05	Silage Stage Forage	0 05	104
SP 0C	Stalk	CONTROL	(<0 01 ppm)
SP 01	Stalk	0 01	77
SP 10	Stalk	0 20	91
ST 0C	Stalk	CONTROL	(<0 01 ppm)

TOTAL NUMBER OF PAGES IS 7 PAGE 05

RESIDUE TEST REPORT

FIELD TEST NUMBER	RI-MV-003-91	PROTOCOL NUMBER.	106-91
REPORT NUMBER.	1	PROJECT NUMBER:	168982

ANALYTICAL SECTION (Continued)

RESULTS

SUMMARY OF METHOD VALIDATION DATA FOR METHOD AG-590

FORTIFIED CORN CONTROL SAMPLE RECOVERY DATA (Continued)

Sample Number	Corn Substrate	Fortification Level (ppm)	% Recovery
ST 01	Stalk	0 01	87
ST 20	Stalk	0 20	80
D.00A	Fodder	0 (Control)	(<0 01 ppm)
D.01A, D.01B	Fodder	0 01	79, 103
D 05A, D 05B	Fodder	0.05	91, 96
D.00B	Fodder	0 (Control)	(<0 01 ppm)
D.10A, D 10B	Fodder	0 10	68, 99
D 20A, D 20B	Fodder	0 20	72, 112
FDP.0C	Fodder	CONTROL	(<0 01 ppm)
FDP.01	Fodder	0 01	78
FDP.05	Fodder	0 05	72
FDT.0C	Fodder	CONTROL	(<0 01 ppm)
FDT.01	Fodder	0 01	75
FDT 05	Fodder	0 05	Rej **
OIL 0	Crude Oil	0 (Control)	(<0 01 ppm)
OIL 01A, OIL 01B	Crude Oil	0 01	Rej **, 87
OIL.05A, OIL 05B	Crude Oil	0 05	84, 86
FLR.0	Flour	0 (Control)	(<0 01 ppm)
FLR.01A, FLR 01B	Flour	0 01	97, 92
FLR.05	Flour	0.05	102
FLR.10	Flour	0.10	85

Results corrected for control values

**Samples analyzed but rejected because of documented problems during workup or analysis

SAMPLES ANALYZED BUT NOT REPORTED

Samples G.00B, G.10A, G 10B, G.20A and G 20B were analyzed by a chemist unfamiliar with the method and recoveries were unacceptable.

Samples FP.0C, FP.10, FP 05, FP.50, FP.IA, FP IB, FP IC, FT 0C, FT 05, FT 50, FT IA, FT IB, and FT IC were samples from the first two sets of Metabolism samples analyzed The substrates were more acidic than previous controls and recoveries were unacceptable due to losses during sample workup The subsequent revision of cleanup procedure, documented in Protocol Amendment #1, allowed for these samples to be analyzed successfully (samples were assigned different code numbers for repeat analysis).

RESIDUE TEST REPORT

FIELD TEST NUMBER. RI-MV-003-91 PROTOCOL NUMBER 106-91

REPORT NUMBER 1 PROJECT NUMBER. 168982

ANALYTICAL SECTION (Continued)

RESULTS

^{14}C-CGA-152005 TREATED CORN RESIDUE DATA

Sample ID	Study Number M91-168-007P Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
	(Injected Phenyl-^{14}C-CGA-152005)				
(Mature Foliage)					
XFP IA	P91400161	0 308	0 032	99	0.032
XFP IB	P91400161	0.308	0 028	104	0.030
XFP IC	P91400161	0.308	0 033 (CV:9%)	96	0.031
(Mature Stalk)					
SP IA	P91400078	0 195	<0 01	103	0.008
SP IB	P91400078	0 195	-NA-**	108	0 007
SP IC	P91400078	0 195	<0.01	108	0 006 (CV·14%)

Sample ID	Study Number M91-168-008P Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
	(Injected Triazine-^{14}C-CGA-152005)				
(Mature Foliage)					
XFT IA	P91400175	1 28	0 14	87	0.15
XFT IB	P91400175	1 28	0 14	90	0 15
XFT IC	P91400175	1 28	0 21 (CV·25%)	94	0 19
(Mature Stalk)					
ST IA	P91400061	0 262	-NA-**	103	-NA-**
ST IB	P91400061	0.262	<0.01	134	0.006
ST IC	P91400061	0 262	<0.01	99	0.006
(Mature Grain)					
GT IA	P91400063	0 038	<0 01	70	<0 001
GT IB	P91400063	0 038	<0.01	70	<0 001
GT IC	P91400063	0 038	<0.01	68	<0 001

*^{14}C incurred levels determined by combustion/LSC by Metabolism Department Reference Lab Notebooks 3955 and 3921

**Sample results not available due to documented problems during workup or analysis.

COMMENTS Results are corrected for procedural recoveries <100%

[CENTERL-DOC RESIDUE] RI-MV-003-91-01 ms/sbh-2/25/92

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-003-91 PROTOCOL NUMBER: 106-91

REPORT NUMBER: 1 PROJECT NUMBER: 168982

ANALYTICAL SECTION (Continued)

RESULTS

^{14}C-CGA-152005 TREATED CORN RESIDUE DATA (Continued)

Sample ID	Study Number 54-91 1 Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
	(Sprayed Phenyl-^{14}C-CGA-152005)				
(0-Day Forage)					
FLP.SB	53434	3 44	1.63	94	1 61
(30-Day Forage)					
FFP SA	53435	0 092	<0 01	97	0 002
FFP SB	53435	"	<0 01	92	0 003
FFP SC	53435	"	<0 01	96	0 002
(46-Day Silage Stage Forage)					
FSP SA	53436	0 034	<0 01	112	<0 001
FSP SB	53436	"	-NA-**	100	-NA-**
(93-Day Mature Fodder)					
FDP SA	53437	0 048	<0 01	54	0 002
FDP.SB	53437	"	<0 01	52	0 001

Sample ID	Study Number 54-91.2 Code No.	Incurred ^{14}C Level (ppm)*	(HPLC) ppm Found	% ^{14}C Extracted	ppm ^{14}C Found in Final Volume
	(Sprayed Triazine-^{14}C-CGA-152005)				
(0-Day Forage)					
FLT SA	53405	3.30	1 69	100	1 30
(30-Day Forage)					
FFT SA	53406	0 029	<0 01	79	0 001
FFT SB	53406	"	<0 01	86	<0 001
(46-Day Silage Stage Forage)					
FST SA	53407	0 048	<0 01	101	0 001
FST SB	53407	"	<0 01	90	0 001
(93-Day Mature Fodder)					
FDT SA	53408	0.009	<0 01	30	<0 001
FDT.SB	53408	"	<0 01	30	<0 001

*^{14}C incurred levels determined by combustion/LSC by Metabolism Department Reference Lab Notebooks 4002 and 4045

**Sample results not available due to documented problems during workup or analysis

COMMENTS Results are corrected for procedural recoveries <100%

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